The influence of freshwater discharge on productivity, microbiota community structure and trophic dynamics in the Murray estuary: evidence of freshwater derived trophic subsidy in the sandy sprat.

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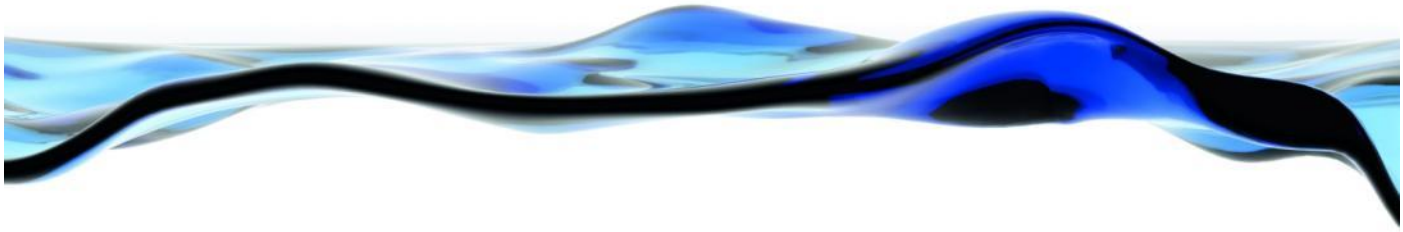

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Executive summary

In the Coorong, at the terminus of the Murray-Darling Basin, the influence of freshwater discharge on water level and salinity regime is generally well understood and in recent years, knowledge of the influence of these factors on biotic patterns and processes has improved. In contrast, understanding of the role of freshwater discharge in promoting ecosystem productivity, through the input of organic matter, is limited. Recent data suggest a potential association between high freshwater discharge, zooplankton species diversity and abundance, and high abundance of a small-bodied (i.e. adult length <100 mm) planktivorous marine fish, sandy sprat (*Hyperlophus vitattus*), in the Murray estuary region of the Coorong. Here we hypothesise that organic matter and biota, transported downstream by freshwater discharge, may be subsidising the diet of sandy sprat and population productivity. As a primary prey item for larger piscivorous fishes, enhanced production of sandy sprat stands to benefit the productivity of higher trophic levels.

The objective of the current study was to investigate the influence of low-volume freshwater discharge in 2014 on water physico-chemistry, primary productivity, microbiota community structure, and the diet and freshwater derived trophic subsidy of sandy sprat. Sampling took place over a series of three events in November–December 2014 across five sites within the Murray estuary and one upstream of Goolwa Barrage. During each occasion samples of water, zooplankton and sandy sprat were collected for analyses of:

- Nutrient concentrations (phosphorus, nitrogen and carbon);
- Phytoplankton abundance and community composition;
- Zooplankton abundance and community composition (quantitative identification and enumeration, and molecular analyses);
- Sandy sprat diet (quantitative identification and enumeration, and molecular analyses of gut content); and
- Stable isotope analyses (SIA) for nitrogen ($\delta^{15}N$), carbon ($\delta^{13}C$) and sulfur ($\delta^{34}S$), including a preliminary evaluation of amino acid-specific SIA for $\delta^{15}N$ and $\delta^{13}C$.

Results of the SIA were also compared to previous measurements for sandy sprat collected from the Coorong under differing hydrological conditions.

Key results

Discharge to the Coorong in 2014 was typically characterised by flows <2000 $ML.day^{-1}$ until late July, when discharge began to increase, peaking at ~23,000 $ML.day^{-1}$ in mid-August. Discharge

was >10,000 ML.day^{-1} for a period of approximately 18 days before decreasing and varying around a mean ~2000 ML.day^{-1} over September–October, before a further reduction to ~1500 ML.day^{-1} during November–December. In association, salinity increased gradually at sampling sites from November to December.

Abiotic and biotic parameters investigated varied both temporally and spatially, typically in association with distance from freshwater discharge points and increasing time from the August flow peak (i.e. from trip 1 to trip 3). Nutrient concentrations and phytoplankton abundance typically decreased with increasing distance from Goolwa and Tauwitchere barrages, and between trip 1 and trip 3, with the exception of some estuarine groups of phytoplankton. Zooplankton abundance also generally decreased over time, while patterns of community structure variability were characterised by greater prevalence of freshwater species at sites closest to Goolwa and Tauwitchere barrages, and increasing dominance of estuarine/marine species across most sites from trip 1 to trip 3. The zooplankton community was less abundant and less diverse than during high discharge in 2010/11, and community structure differed from a period of low discharge in 2003, which followed an extended period (>600 days) of no discharge, due to greater relative abundances of freshwater copepods and freshwater/estuarine rotifers, in 2003 and 2014, respectively. Spatio-temporal variability in zooplankton community structure in November–December 2014 was reflected in the diet of sandy sprat.

The diet of sandy sprat was variable, but estuarine harpacticoid copepods were the dominant prey item. The freshwater rotifer *Keratella australis* and cladoceran *Bosmina meridionalis* also comprised significant proportions of the diet at some sites, and when present, were selectively preyed upon. The prevalence of these freshwater species in the gut content of sandy sprat generally decreased as freshwater discharge decreased, suggesting a direct link between freshwater discharge and trophic subsidy of sandy sprat.

Sandy sprat δ^{13}C became progressively more enriched (less negative) from trip 1 (mean = –20.4‰) to trip 3 (–19.4‰) but δ^{34}S exhibited the inverse pattern (from 15.6‰ to 14.8‰). As discharge was relatively constant between the three trips, these patterns suggest a large input of freshwater-derived organic matter (and usage by sandy sprat) with the unregulated August 2014 flow event, with isotopic signatures gradually re-equilibrating to a 'Coorong' organic matter signature over time. Sandy sprat δ^{13}C signatures were similar in 2013 (–19.8‰) and 2014, years of similar freshwater discharge. However, δ^{13}C was substantially enriched (–17.7‰) and δ^{34}S depleted (11.4‰) in 2007, when freshwater discharge was minimal. Thus, there was an association between sprat isotopic signatures and freshwater discharge and this pattern is consistent with a greater usage of freshwater-derived organic matter following higher flows.

Preliminary measurements using amino acid-specific SIA were also consistent with some incorporation of freshwater-derived organic matter in the Coorong food web.

Conclusion

This is the first study to demonstrate that organic matter and biota exported to the Murray estuary with freshwater discharge through the Murray Barrages contributes materially to estuarine productivity. As such it presents empirical data to directly inform and support the delivery of environmental water allocations to the Coorong on the basis of supporting trophic dynamics. Whilst the results are specific to the 2014 hydrograph, they suggest that even low–volume discharge can have measurable benefits for trophic dynamics, whilst conspicuous flow pulses (~20,000 $ML.day^{-1}$) may provide productivity benefits that last for periods of months following flow recession.

1 Introduction

Estuaries represent the dynamic interface and conduit between freshwater and marine environments. The interplay between freshwater discharge, weather and tidal cycle dictates the physical and chemical nature of estuaries, influencing connectivity between freshwater, estuarine and marine environments, and the estuarine salinity regime. These factors subsequently influence the distribution and abundance of estuarine biota (Elliott and Whitfield 2011). Freshwater discharge also transports organic matter and biota of freshwater origin to the estuarine environment, potentially subsidising estuarine food webs (Darnaude *et al.* 2004, Wissel and Fry 2005). Reductions in freshwater discharge to estuaries can result in habitat fragmentation, altered salinity regimes and reduced productivity. The use of environmental water allocations is becoming increasingly common to achieve ecological benefits in estuaries of regulated rivers (Adams 2014). Nonetheless, ecologically effective use of these allocations is reliant on knowledge of the association of freshwater discharge with key ecosystem processes (Arthington *et al.* 2006).

The Coorong, in south-eastern Australia, is situated at the terminus of the Murray-Darling Basin (MDB), the nation's longest river system. The MDB is highly regulated, with catchment inflows largely dictated by releases from several large headwater dams. Discharge to the Coorong is further regulated by a series of five barrages that separate the Coorong and the freshwater Lower Lakes. Regulation and consumptive water use have dramatically altered the hydrology of the MDB. As a result, on average, only ~39% (4723 GL) of the natural mean annual discharge (12,233 GL) now reaches the Coorong (CSIRO 2008). Furthermore, the frequency of periods of no freshwater discharge to the Coorong has increased dramatically. Reduced discharge has led generally to elevated salinities throughout the Coorong and the barrages themselves represent distinct physical barriers to the movement of biota between estuarine and freshwater environments. There is improving understanding of the impact of these fundamental ecosystem changes on a range of biota (e.g. migratory waders, fish, etc.) (e.g. Paton *et al.* 2009, Zampatti *et al.* 2010), but knowledge of changes to estuarine productivity as a result of river regulation remains poor.

As a Ramsar listed wetland of international importance and Icon Site under the Murray-Darling Basin Authority's *The Living Murray Program*, the Coorong is now the subject of substantial ecosystem rehabilitation effort. Numerous monitoring and research programs have been undertaken in the past two decades investigating various species/ecosystem patterns and processes (e.g. Brookes *et al.* 2009, Zampatti *et al.* 2012, Oliver *et al.* 2014, Paton and Bailey

2014), with the aim of informing and supporting ecosystem management. To date, these programs have supported the implementation of various management interventions including hydrological restoration (i.e. environmental water delivery), revegetation and restoration of hydrological connectivity (e.g. fishway construction) (DEH 2010). Nonetheless, there remains a need to better understand ecosystem function in relation to freshwater discharge to inform and justify the delivery of environmental water. Many of the aforementioned monitoring/research programs were undertaken post 2006, during a period characterised by hydrological extremes, including a period of no freshwater discharge to the Coorong from 2007–2010 during the Millennium drought and subsequent high discharge during 2010–2012. As such, these studies provide a basis for assessing synergies in biotic patterns and generating testable hypotheses on ecosystem function in relation to freshwater discharge.

Developing a greater understanding of ecosystem function in relation to freshwater discharge is fundamental to informing the Environmental Watering Plan under the Murray-Darling Basin Plan. The plan aims to recover 2,750 GL of surface water to achieve environmental benefits in the MDB, which is to be delivered as guided by the Environmental Watering Plan. The Lower Lakes and Coorong represent a key site for the delivery of environmental water under the Basin Plan, but incorporation of flow requirements into the Environmental Watering Plan requires the support of robust science to justify environmental water delivery.

1.1 Sandy sprat as an ecological indicator

Estuarine fish are iconic and useful indicators of ecosystem function and altered flow regimes (Sheaves *et al.* 2012). Highly variable freshwater discharge to the Coorong from 2006 to 2013 was accompanied by substantial variability in the abundance of some fish species (Ye *et al.* 2012, Bice and Zampatti 2014). Throughout this period, sandy sprat (*Hyperlophus vitattus*), a small-bodied (adult total length <100 mm), pelagic member of the Clupeidae, was found to be the most abundant species in the Murray estuary region of the Coorong. However, the species was least abundant from 2007–2010 when no freshwater was discharged to the Coorong and most abundant in 2011/12, following a prolonged period of high freshwater discharge (Bice and Zampatti 2014) (Figure 1). This pattern highlights an association between abundance and freshwater discharge. The species is typically considered a marine migrant, which spawns in the marine environment but utilises estuaries as feeding and nursery habitats (Gaughan *et al.* 1996), and is common across much of southern Australia (Gomon *et al.* 2008).

Accompanying increased abundance of sandy sprat in association with elevated freshwater discharge to the Coorong, high discharge in 2010/11 was also associated with high nutrient

concentrations and increased phytoplankton and zooplankton diversity and abundance (Seuront and Leterme 2010, Aldridge and Brookes 2011, Shiel and Aldridge 2011, Oliver *et al.* 2014). As a planktivorous fish species, sandy sprat may forage upon freshwater zooplankton transported from upstream and/or take advantage of increased estuarine productivity as a result of freshwater inputs of carbon and nitrogen. As such, sandy sprat abundance in the Murray estuary may in part be influenced by productivity and food availability. Attempts have been made to characterise the food-web of the Coorong, but these occurred during a period of very low or no freshwater discharge, so the role of freshwater discharge in subsidising the trophic web of the Coorong is poorly understood (Lamontagne *et al.* 2007, Deegan *et al.* 2010).

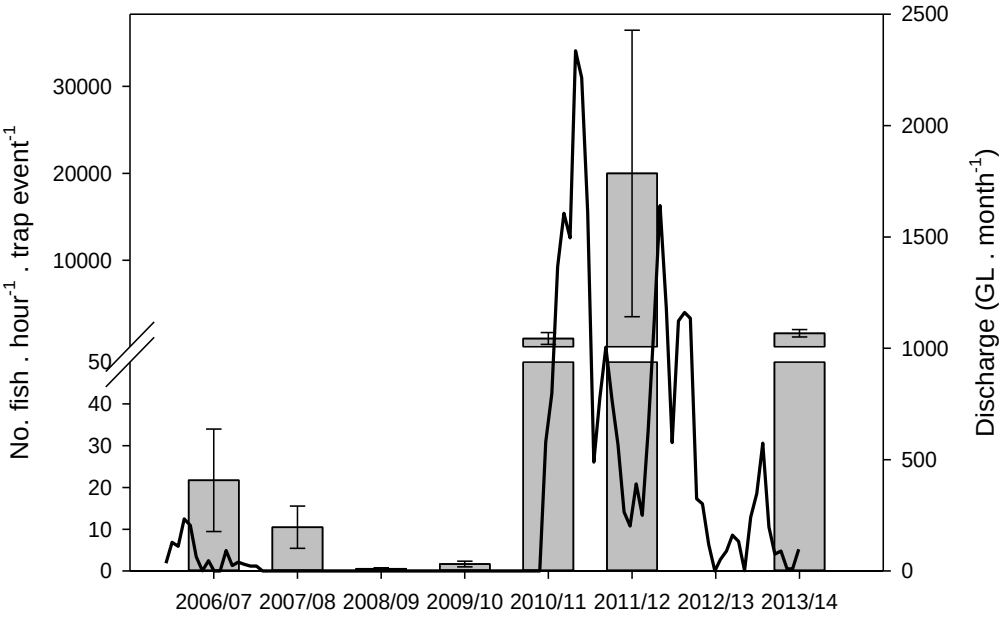

Figure 1. Mean abundance of sandy sprat (fish.hr^{-1}.trap event^{-1} ± SE) downstream of Tauwitchere Barrrage in the Murray estuary from 2006–2014 (bars), with monthly total barrage discharge (GL.month^{-1}) overlaid (line). Abundance data from Bice and Zampatti (2014). No sampling was conducted in 2012/13. Discharge data sourced from DEWNR.

Sandy sprat is highly important in the trophic dynamics of the Coorong. Whilst not explicitly studied within the Coorong, the species is an important prey item for piscivorous birds (e.g. tern species) across other areas of southern Australia (Klomp and Wooller 1988, Taylor and Roe 2004), and this importance likely extends to the Murray estuary region of the Coorong. Furthermore, recent evidence suggests sandy sprat is preyed upon by several larger piscivorous fish species within the Murray estuary, including both Australian salmon (*Arripis truttaceaus*) and juvenile mulloway (*Argyrosomus japonicas*; <400 mm length), for which the species was among the most

important prey items (Giatas and Ye 2015). Subsequently, factors influencing the abundance of sandy sprat may also affect the abundance of higher trophic levels, including commercially and recreationally important fish species, through trophic interactions.

Empirical data on the influence of freshwater discharge on key ecological patterns and processes is vital to inform and justify the delivery of environmental water to the Coorong. Large floods in the lower River Murray, which correspond to periods of high freshwater discharge to the Coorong (e.g. > 50,000ML.day^{-1}) are largely unaffected by regulation, whilst periods of low to medium freshwater discharge (2,000–50,000 ML.day^{-1}) are generally most affected (Maheshwari *et al.* 1995). Low to medium volume flows are also those most likely to be reinstated through environmental water allocation under the Murray-Darling Basin Plan. Elucidating the role of freshwater flows of this magnitude in subsidising the trophic web of the Coorong would provide a strong basis for the delivery of low-volume freshwater flows to the Coorong through the use of environmental water allocations.

1.2 Objectives

The objective of this study is to investigate the influence of low-volume freshwater discharge (<25,000 ML.day^{-1}) on productivity (i.e. phytoplankton community structure and biomass), microbiota community structure and trophic dynamics in the Murray estuary region of the Coorong. Specifically, the study will utilise data collected in 2014 and data from past studies, and a range of different methods to investigate;

1) Spatio-temporal variability in primary productivity, as inferred by phytoplankton community structure and abundance;
2) Spatio-temporal variability in microbiota (e.g. zooplankton) community structure and abundance;
3) Spatio-temporal variability in the abundance and diet of sandy sprat; and
4) Evidence of freshwater derived trophic subsidy by using sandy sprat as an indicator species and integrating the above data with stable isotope analyses.

The findings of the current study will address key knowledge gaps and improve understanding of the influence of low-volume freshwater discharge on biotic community structure and trophic structure in the Coorong (Deegan *et al.* 2010, Bice *et al.* 2014). Furthermore, the study will provide empirical data on the ecological benefits of low-volume freshwater discharge to the Coorong to support future environmental water planning and delivery, including implementation of the Basin Environmental Watering Plan.

2 Methods

2.1 Study region

This study was undertaken in the Coorong, at the terminus of Australia's longest river system, the Murray-Darling Basin (Figure 2). The River Murray flows into the expansive Lake Alexandrina before discharging into the Coorong through five major flow paths at Goolwa, Mundoo Channel, Boundary Creek, Ewe Island and Tauwitchere. The Coorong, is a narrow (2–3 km wide) inter–dune estuarine lagoon that runs in a south-easterly direction from the Murray Mouth for ~140 km. Typically, it is divided into three major regions; the Murray estuary: from Goolwa Barrage to Pelican Point, the North Lagoon: from Pelican Point to Parnka Point, and the South Lagoon: from Parnka Point to the south-eastern end of the Coorong.

In the 1930's, tidal barrages were constructed across all five flow paths, significantly reducing the extent of the estuary and creating a distinct ecological barrier between marine/estuarine and freshwater environments. Flows to the Coorong are now controlled by the 7.6 km barrage network, with water released through a series of 593 gated bays. Under natural conditions (pre-1930s), mean annual discharge was ~12,233 GL but there was strong inter-annual variation (Puckridge *et al.* 1998), whilst under regulated conditions, average annual end-of-system discharge has been reduced to ~4723 $GL.y^{-1}$ (CSIRO 2008). Discharge over the last two decades has been highly variable and was characterised by a prolonged period of low flow from 1997–2010, including a three-year period of zero discharge (March 2007–August 2010) (Figure 3). Discharge increased abruptly in September 2010 and annual discharges in 2010/11, 2011/12 and 2012/13 were approximately 12,500, 8800 and 5200 GL, respectively. Annual discharge decreased in 2013/14 to~1600 GL and further so in 2014/15 to ~860GL.

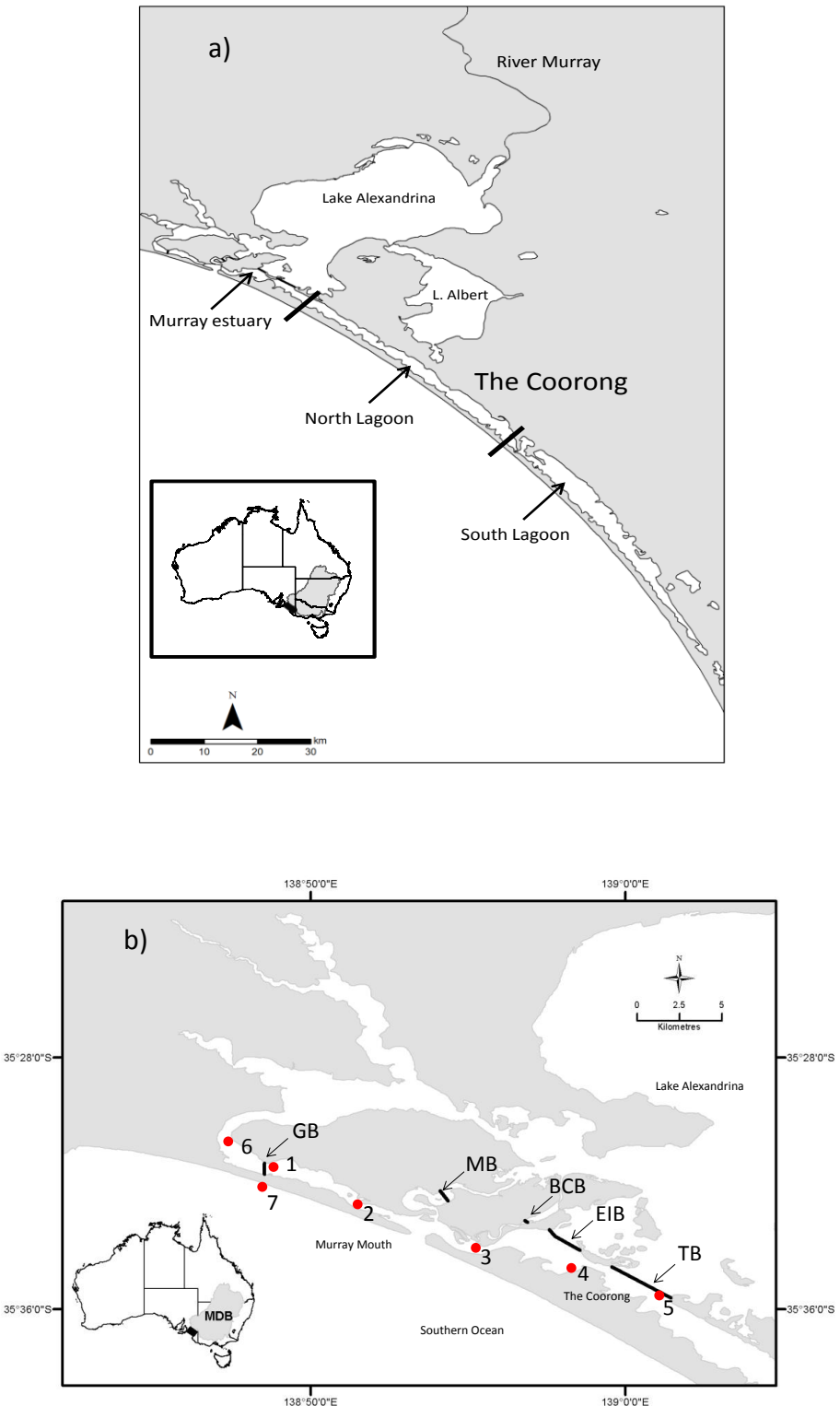

Figure 2. a) Map of the Coorong and Lower Lakes, indicating the geographical division (black bars) of the Coorong into the Murray estuary, North Lagoon and South Lagoon, and b) the Murray estuary region of the Coorong showing the location of specific sampling sites 1–7. Barrages are represented by bold lines. GB = Goolwa Barrage, MB = Mundoo Barrage, BCB = Boundary Creek Barrage, EIB = Ewe Island Barrage and TB = Tauwitchere Barrage.

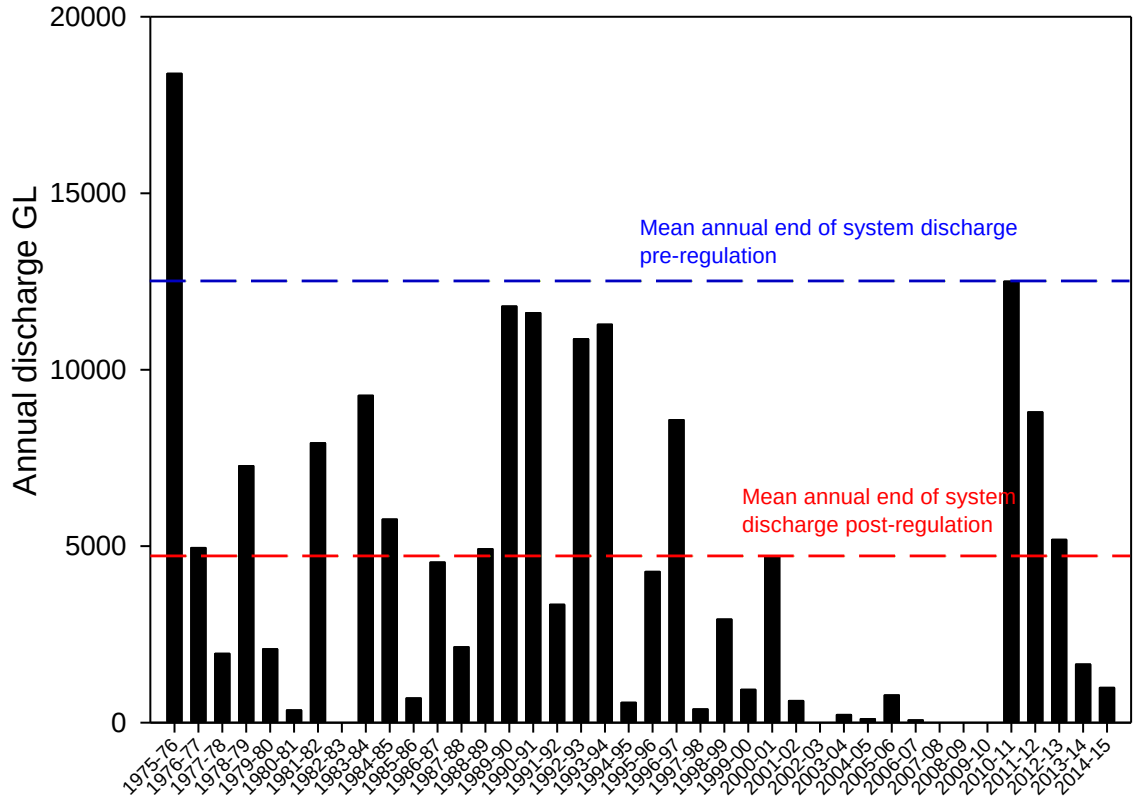

Figure 3. Annual discharge (GL) from the Murray Barrages to the Coorong from 1975/76–2014/15. Mean annual discharge prior to regulation (pre-1930s; dashed blue line) and post regulation (post-1930s; dashed red line) are also presented.

The Coorong exhibits a strong salinity gradient, with salinity increasing in a south-easterly direction and is primarily influenced by freshwater discharge through the barrages, marine tides and evaporation. During times of low flow, salinity in the Murray estuary region typically reflects that of seawater (~35 $g.L^{-1}$), but gradually increases to hypermarine (>100 $g.L^{-1}$) through the North and South Lagoons (Geddes 1987). Conversely during high freshwater discharge, salinity within the Murray estuary and even the North Lagoon can range between fresh and marine (1 – 35 $g.L^{-1}$), with reductions in salinity in the South Lagoon also noted (Geddes 1987).

This study is primarily focused on the Murray estuary region as the predicted hydrograph for barrage discharge in spring/summer 2014 indicated low-volume discharge was likely, and thus the area likely to be influenced by freshwater discharge (e.g. reduced salinities) would be confined to this region. The Murray estuary is also the region of the Coorong where sandy sprat is typically found in highest abundance (Ye *et al.* 2012).

2.2 Field sampling

Sampling was conducted at five sites (Figure 2b and Table 1) in the Murray estuary (sites 1–5) over three sampling events: 1) 5–7 November; 2) 25–27 November; and 3) 17–19 December 2014. Sampling was conducted to collect: 1) integrated water samples; 2) zooplankton; and 3) sandy sprat. Additional sampling was undertaken to collect: 1) integrated water samples; and 2) zooplankton from upstream of Goolwa Barrage (site 6) during trip 2 (Figure 2b and Table 1). Additional sampling was also undertaken during trip 3 at a series of locations in Encounter Bay (site 7) with the aim of collecting samples of sandy sprat from marine habitats outside the Coorong (Figure 2b and Table 1).

Table 1. Details of sites sampled in the current project including site number, name, latitude and longitude, and the sampling trips during which they were sampled.

Site No.	Site name	Latitude	Longitude	Trips sampled
1	Downstream Goolwa Barrage	35˚31'24.16" S	138˚48'33.79"E	1–3
2	Rushy Island	35˚32'22.50" S	138˚50'52.67"E	1–3
3	Godfrey's landing	35˚33'52.78" S	138˚54'18.63"E	1–3
4	Ewe Island	35˚34'15.65" S	138˚50'52.67"E	1–3
5	Downstream Tauwitchere Barrage	35˚35'23.60" S	139˚00'56.30"E	1–3
6	Upstream Goolwa Barrage	35˚30'35.88" S	138˚47'14.70"E	2
7	Encounter Bay*	-	-	3

*NOTE. Several sub-sites were sampled in Encounter Bay

Water samples

Water samples were collected for analyses of carbon and nutrients (i.e. nitrogen and phosphorus), phytoplankton community structure and abundance. During each sampling event, a 3 m PVC tube was used to collect an integrated water sample from the surface to a depth just above the sediments. The tube was lowered vertically into the water column until it spanned the depth, then the top was closed and the tube carefully raised and the open end lifted from the water and placed in a bucket to capture the sample. Different sub-samples of the integrated water sample were preserved on ice for analyses of carbon and nutrients, and preserved with Lugol's iodine, in the case of phytoplankton identification and enumeration. Water samples were taken from sites 1–6 during all sampling events.

Zooplankton

Samples of the zooplankton community were collected for three purposes: 1) for quantitative identification and enumeration to determine species composition and abundance, 2) for molecular analyses as a complementary technique for determining species composition and abundance, and 3) stable isotope analyses (SIA).

Zooplankton samples for quantitative identification and enumeration were collected using a 4 L Haney trap. During each sampling event, three independent replicates were taken from each site from spatially separated locations (>20 metres apart). Each replicate consisted of a composite of three trap samples taken from the surface, middle and bottom of the water column. All samples were concentrated using a 35 µm plankton net to approximately 20 mL of sample, preserved with 95% ethanol, and returned to the lab. A fourth sample was collected using the same technique, from which a sub-sample was taken for community composition and abundance analyses using molecular techniques. Samples were also collected with a 35 µm plankton net from within the top 1 m of water within the pelagic zone to assist with species identification.

Additional net hauls were undertaken to collect samples for SIA using a suite of plankton nets (mesh sizes 35–500 µm). Sampling continued for up to a maximum of 30 minutes or until an adequate sample (>0.4 g wet weight) had been collected. Samples were initially preserved on ice, before being concentrated and frozen at the first possible opportunity (generally <6hrs from collection).

Sandy sprat

Samples of sandy sprat were collected for three purposes: 1) investigation of diet using quantitative identification and enumeration of gut content; 2) investigation of diet using molecular analyses of gut content; and 3) SIA. Samples of sandy sprat were collected using a 61 m long and 2 m deep seine net, which consisted of two 29 m-long wings (22 mm mesh) and a 3 m-long bunt (8 mm). The net was deployed in a semi-circle and hauled onto shore (Figure 4). Sandy sprat were sorted from all other fish species, a sub-sample of up to 60 individuals measured for length (mm, caudal fork length (FL)) and where possible ≥ 100 individuals collected for various analyses (i.e. quantitative identification and enumeration of gut content $n \geq 10$, molecular analyses of gut content $n \geq 10$, SIA and amino acid analyses $n \geq 80$). All remaining fish species were returned to the water. Net hauls were conducted until an adequate sample of sandy sprat was obtained (1–8 hauls). Sandy sprat samples for gut content analyses were preserved in ethanol (75%), whilst samples for molecular analyses were initially stored on ice and frozen at the

nearest opportunity. Samples for SIA were thoroughly washed with distilled water in the field and preserved on ice and later frozen. Sampling of sandy sprat was undertaken at sites 1–5 during each sampling trip, and at site 7 only during trip 3.

Figure 4. The seine netting method used to sample sandy sprat from the Murray estuary region of the Coorong.

The above method was not used to assess abundance of sandy sprat. Instead, data on inter- and intra-annual variability in sandy sprat abundance was obtained from two allied fish sampling programs as outlined below.

Inter- and intra-annual variability in sandy sprat abundance

Abundance data for sandy sprat in the Murray estuary was gathered from two fish monitoring programs funded under the Murray-Darling Basin Authority's (MDBA) *The Living Murray Program* (TLM) (Ye *et al.* 2013, Bice and Zampatti 2014).

Project One, sampled fish at the Murray Barrages and associated fishways to inform barrage operation, and includes regular sampling of fish at two sites shared with the current project, 1) downstream Goolwa Barrage and 2) downstream Tauwitchere Barrage (see Bice and Zampatti 2014 for specific detail). These sites have typically been sampled monthly from October to January annually since 2006. Sampling in 2014/15 was undertaken on 30th October 2014, 26th November 2014, 17th December 2014 and 29th January 2015. Data from this project was used to investigate inter- and intra-annual variability in sandy sprat abundance at these sites. Differences in the relative abundance of sandy sprat (fish.hour^{-1}.trap event^{-1}) sampled between years at both

sites were analysed using uni-variate single-factor PERMANOVA (permutational ANOVA and MANOVA), in the software package PRIMER v. 6.1.12 and PERMANOVA+ (Anderson *et al.* 2008). These analyses were performed on fourth-root transformed relative abundance data and Euclidean distance resemblance matrices (Anderson *et al.* 2008). To allow for multiple comparisons between years at each site, a false discovery rate (FDR) procedure presented by Benjamini and Yekutieli (2001), hereafter the 'B–Y method' correction, was adopted ($\alpha = \sum_{i=1}^{n}(1/i)$; e.g. for $n_{comparisons}$ = 15, B-Y method α = 0.05/ (1/1 + 1/2 + 1/3…….+1/15) = 0.015) (Benjamini and Yekutieli 2001, Narum 2006).

Project Two (Ye *et al.* 2013), involved sampling which primarily targeted key estuarine fish species to determine patterns in abundance and population demographics against TLM Icon Site targets. It includes sampling of two sites shared with the current project, 1) downstream Tauwitchere Barrage and 2) Godfrey's Landing, and a third site (i.e. Beacon 19), which is situated between site 1 (downstream Goolwa barrage) and site 2 (Rushy Island) of the current study. This sampling involved three replicate hauls of a seine net, using the same net and method detailed above for sandy sprat sample collection. All species were identified and enumerated. Sampling for this project in 2014/15 was undertaken on 12–13 November 2014, 8–10 December 2014 and 10–12 February 2015. Data from this project are described qualitatively and are used to provide supporting information to Project One, on intra-annual variability in sandy sprat abundance in 2014/15.

2.3 Water quality and phytoplankton community structure

Whole water samples and GF/C filtered (glass microfiber filters) water samples were stored frozen prior to analyses by the Australian Water Quality Centre (National Association of Testing Laboratories registered) using their standard methods. Complete water samples were analysed for Total Phosphorus (TP), Total Organic Carbon (TOC) and Total Kjeldahl Nitrogen (TKN), while filtered samples were analysed for Filterable Reactive Phosphorus (FRP), Nitrate + Nitrite (NOx) and ammonium-N (NH_4). Results are reported correspondingly as concentrations of P, N and C. Chlorophyll concentrations were determined spectrophotometrically following ethanol extraction of the GF/C filters. Known volumes of water samples fixed with Lugol's iodine were left to stand overnight and the settled phytoplankton identified and enumerated microscopically by an external provider (AlgaeTest Consulting). Spatio-temporal variability in phytoplankton community composition between trips and sites was assessed graphically using Non-Metric Multi-Dimensional Scaling (MDS) ordination. The MDS was generated from a Bray-Curtis similarity matrix of untransformed average site phytoplankton counts.

2.4 Microbiota/zooplankton community structure

Quantitative identification and enumeration

In the laboratory, quantitative samples were inverted three times and a 1 mL sub-sample was transferred into a pyrex gridded Sedgewick-Rafter cell. The entire sub-sample was counted and zooplankton identified using a Nikon diaphot compound microscope. This was repeated three times for each sample. The average abundance of each species and the total abundance of zooplankton were then calculated and expressed as numbers of individuals per litre (ind.L^{-1}). All zooplankton were identified to species level where possible using published descriptions (Bayly 1992, Koste 1978, Shiel 1995, Smirnov and Timms 1983). The proportional contributions of each microbiota taxa identified to community composition were then calculated using the average taxa and total community abundance from all sites and trips. Zooplankton biomass was also calculated for the one off sampling at site 6 by multiplying the average number of each species per volume by the species dry weight. Dry weight estimates were obtained from the literature for the identified species (Dumont *et al.* 1975, Pauli 1989, Masundire 1994, Sendacz *et al.* 2006, Dagne *et al.* 2008). If estimates were not available for a particular species, a species of similar size and/or genus was used.

Differences in the total abundance (ind.L^{-1}) of zooplankton (all species combined), sampled between trips and sites was analysed using two-factor (i.e. trip and site) uni-variate PERMANOVA (Anderson *et al.* 2008). These analyses were performed on fourth-root transformed relative abundance data and Euclidean distance resemblance matrices (Anderson *et al.* 2008). Spatio-temporal variability in the composition of the zooplankton community (i.e. species identity and abundance) among sites and across trips was assessed graphically using MDS, whilst two-factor (i.e. trip and site) multi-variate PERMANOVA was used to test for significant differences in community composition. These analyses were performed on Bray-Curtis similarity matrices of fourth-root transformed relative abundance data (ind.L^{-1}). The low number of samples collected for these analyses resulted in low numbers of unique permutations for both sets of analyses, and thus, Monte-Carlo *p*–values are presented (Anderson *et al.* 2008). Furthermore, no correction for significance was applied, but rather $\alpha = 0.05$ was retained. When significant differences occurred between pairwise comparisons of community composition, a similarity percentages (SIMPER) analysis was undertaken to identify species contributing to these differences. A 40% cumulative contribution cut-off was applied.

Molecular analyses

A number of different sampling techniques were used in order to obtain representative coverage of the broad size range of aquatic microeukaryotes likely to be present, from single celled phytoplankton to large zooplankton. Integrated water samples from the top 2–3 m of the water column were GF/C filtered to collect organisms above >1 µm in size. As the volume that could be filtered was relatively small, only common organisms were collected in this way and tended to be the smallest microbes. Zooplankton net and trap sampling was aimed at collecting zooplankton and other small multicellular biota >35 µm, methods for which are detailed in Section 2.2.

All field samples were freeze dried prior to molecular analyses, then DNA was extracted using the PowerSoil DNA isolation kit (Mo Bio) and sub-samples of equivalent concentration prepared for further analyses. A fragment of the 18S rRNA gene that is conserved in eukaryotes was amplified using the Polymerase Chain Reaction (PCR) and sequenced using the Illumina MiSeq platform. PCR amplification and MiSeq sequencing was performed by the Australian Genome Research Facility, Brisbane, Australia. Quality checked, full length sequences were sorted by abundance, singletons were removed, chimeras filtered out and the remaining sequences combined into operational taxonomic units (OTUs) based on a minimum sequence similarity of 97%. Taxonomy was assigned using Qiime by referencing the Silva database (Version Silva_119). This method does not include bacteria and so exclude the cyanobacteria.

The molecular data was not analysed based on sequence number as volume manipulations, differences between PCR responses of particular organisms, and different and unknown gene copy numbers make quantitative analyses difficult. Instead the OTU results of each sample were expressed in terms of percentage contributions to the community composition in that sample and then percentage contributions were compared to identify changes between sampling sites, sampling trips and the field community and the fish gut content. Differences between sites and trips were identified by calculating Bray-Curtis similarities between samples, and using MDS for their ordination in PRIMER v6 (Clarke and Gorley 2006). Testing for statistically significant differences was carried out by permutational analysis of variance (PERMANOVA+ for PRIMER, Anderson et al. 2008). The B-Y method significance correction was applied when multiple comparisons were undertaken (Benjamini and Yekutieli 2001, Narum 2006). When significant differences occurred between pairwise comparisons of community composition, SIMPER analysis was undertaken to OTU's contributing to these differences. A 40% cumulative contribution cut-off was applied. All data were square root transformed prior to analyses.

2.5 Sandy sprat diet

Gut content – identification and enumeration

A minimum of ten fish were selected from each site per trip for dietary analysis via identification and enumeration of gut content. For each fish, the gastrointestinal tract was extracted and opened under an Olympus SZH10 stereozoom microscope. The content of the gut was then extracted and examined under a Nikon diaphot compound microscope. Prey items were identified to the highest taxonomic level possible and enumerated. This was repeated until five fish from each site per trip had been processed that contained prey items identifiable to at least the taxonomic level of order.

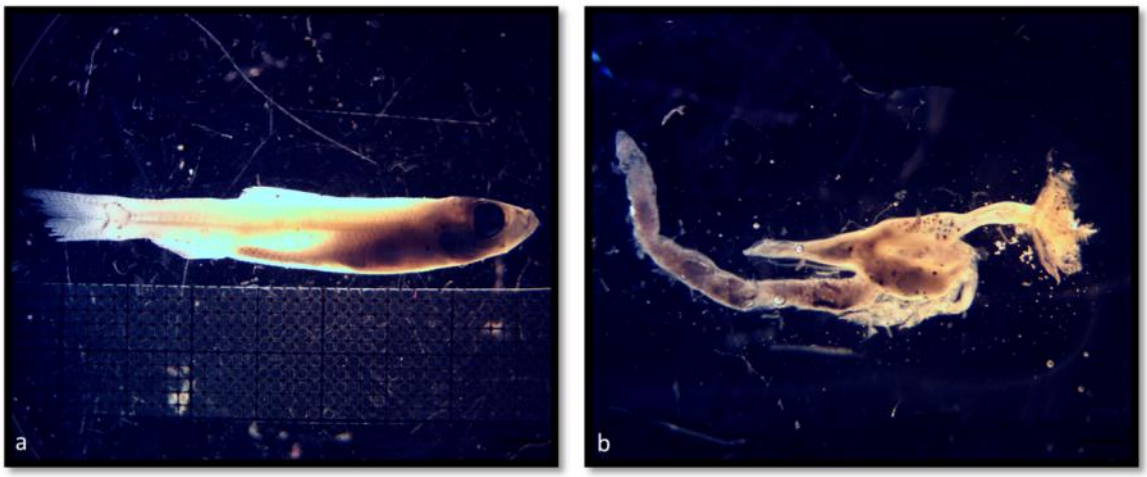

Figure 5. a) sandy sprat (~50 mm FL) prior to dissection and (b) an extracted gastrointestinal tract prior to opening.

Diet was examined by calculating the number of items of a given prey taxa within the esophagus and stomach, which was then expressed as a percentage of the total number of food items. The mean number of total prey items (all species combined) found in fish guts was compared between trips and sites using two-factor uni-variate PERMANOVA, performed on a Euclidean distance similarity matrix. Statistical difference in the composition of gut content (i.e. taxa identity and proportional contribution) between sites and trips was investigated with two-factor multi-variate PERMANOVA. Proportion data was arcsine transformed prior to analyses. The B-Y method significance correction was applied when multiple comparisons were undertaken (Benjamini and Yekutieli 2001, Narum 2006). When significant differences in gut content occurred, SIMPER analysis was undertaken to identify species contributing to these differences. A 40% cumulative contribution cut-off was applied.

Prey item selectivity was investigated by calculating the Strauss index of food selectivity (Strauss 1979):

$$L = r_i - p_i$$

where L is the Strauss index of food selectivity, r_i is the relative abundance of a food item in the diet (proportion of total number in diet) and p_i is the relative abundance of the food item in the habitat (proportion of total catch). Possible values range from +1, which indicates perfect selection for a prey type, and –1, which indicates perfect selection against a prey type. The Strauss index for food selectivity was calculated for each individual prey type at each site for each trip.

Gut content – molecular analyses

A sub-sample of ten sandy sprat from each site per trip were individually wrapped in alfoil and frozen in the field until analysed. In the laboratory, each fish was individually placed on a fresh piece of laboratory tissue paper under a dissecting microscope and the full intestinal tract removed using sterile laboratory dissecting needles and needle nose tweezers. All fish muscle tissue was removed from the intestinal tract by gently rolling and sliding it over the tissue before placing it in a disposable polystyrene weighing boat. The intact intestine was cut open and the contents scraped to one corner of the weighing boat before being washed into a 1.5 ml Eppendorf microcentrifuge tube with a minimum amount of distilled water. DNA was extracted from these samples using Qiagen's Tissue and Blood DNA kit. Further preparation steps, PCR protocols and amplicon sequencing then followed those described above in Section 2.4.

The extracted fish gut sequences were compared with the curated SILVA database (see Section 2.4), but crustacean sequences were further compared with the significantly larger, but non-curated GenBank database, to provide potentially greater taxonomic resolution. The 'identification' of an OTU depends on having a sample sequence of sufficient length and reliability, and the sequence of the particular organism being reliably represented in the database. Development of genetic sequence databases remains a work in progress and thus, alignment of sampled sequences with database sequences are often 'closest' comparisons (taxonomically) and should not be considered definitive.

In order to investigate major biotic groups contributing to the diet only OTU's that contributed >1% of OTU composition in the gut were considered in analyses. Differences in fish diets, based on OTU composition, between sites and trips were identified by calculating Bray-Curtis similarities between samples, and using MDS for their ordination in PRIMER v6 (Clarke and Gorley

2006). Testing for statistically significant differences was carried out by permutational analysis of variance (PERMANOVA+ for PRIMER, Anderson *et al.* 2008). The B-Y method significance correction was applied when multiple comparisons were undertaken (Benjamini and Yekutieli 2001, Narum 2006). When significant differences in gut content OTU composition occurred, SIMPER analysis was undertaken to identify species contributing to these differences. A 40% cumulative contribution cut-off was applied. To simplify these analyses, SIMPER was performed only for prey items that constituted >2.5% of OTU composition at a site during any trip.

2.6 Trophic subsidy

A primer on stable isotope analyses

Part of the assessment undertaken in this study involves the use of SIA. Carbon (C), nitrogen (N) and sulfur (S) isotopic ratios have been widely used to evaluate the origin of organic matter driving estuarine food-webs and to quantify the number of 'links' (or trophic levels) in estuarine food webs (Peterson and Fry 1987, Darnaude *et al.* 2004, Connolly *et al.* 2009). In brief, C, N and S have several stable isotopes that vary in molecular weight as a function of the number of neutrons in their nucleus. Depending on how the organic matter is produced (e.g. C3 vs C4 plants, etc.) and the cycling of elements in the ecosystem (denitrification, sulfate reduction, etc.), organic matter can have different contents for heavy vs lighter isotopes for a given element. In ecological studies, this is usually evaluated through the ratio of $^{13}C/^{12}C$, $^{15}N/^{14}N$ and $^{34}S/^{32}S$ for carbon, nitrogen and sulfur, respectively. These isotope ratios are usually expressed using the δ ('del') notation ($\delta^{13}C$, $\delta\ ^{15}N$ and $\delta\ ^{34}S$). Peterson and Fry (1987) provide a detailed summary of the key factors influencing C, N and S isotopic ratios in food-webs and the rationale for expressing results using the del notation.

SIA is a useful approach for investigating food webs and trophic dynamics of fishes, as the isotopic signatures of fish muscle tissue can reflect their diet over a much longer period of time (probably weeks to months in the case of sandy sprat) relative to gut content analysis (probably hours in the case of sandy sprat). SIA provides two broad types of information when evaluating fish diet. Firstly, because isotopic ratios of $\delta^{15}N$ increase in a predictable fashion between predator and prey, the trophic level of predators can be reliably quantified (Vander Zanden and Rasmussen 1999, 2001). Secondly, and of most relevance to this study, SIA can provide insight on the origin of organic matter used by fish. In particular, $\delta^{13}C$ from freshwater sources tends to have a depleted signature (i.e. 'more negative'), whilst marine sources tend to be have enriched signatures (i.e. 'more positive'). An additional advantage of SIA in estuaries is that $\delta\ ^{34}S$ can be a

reliable indicator for organic matter produced in the estuary (typically 'depleted') as opposed to organic matter imported from outside the estuary (typically 'enriched') (Peterson and Fry 1987, Fry and Chumchal 2011).

A disadvantage of traditional bulk SIA analysis is that the isotopic signature of fish and of their prey must both be measured. This can be problematic for plankton-eating fish because collecting enough zooplankton for SIA analysis can be difficult. To alleviate this problem, compound-specific SIA (CSIA), using amino acids, has been recently developed (see McClelland and Montoya 2002, Chikaraishi *et al.* 2009, Larsen *et al.* 2009). In brief, amino acids are the essential building blocks for proteins, but some can only be produced by plants and certain microorganisms, and are thus preserved along the food chain. Therefore, it is possible to deduce the origin of organic matter consumed by a fish by looking at the isotopic signature for selected amino acids in that fish only. However, this technique is more labour-intensive than traditional bulk SIA and is yet to be trialled in semi-arid estuaries. CSIA was trialled here in parallel with traditional SIA.

Stable isotope analyses

SIA was undertaken on samples of both sandy sprat and zooplankton. Samples of sandy sprat and zooplankton were thawed and any organic matter debris was removed. For sandy sprat, dorsal muscle from several fish was removed and combined to produce a ~2 g wet weight sample for each replicate. Where possible, up to five replicates were prepared per site and trip. In the case of zooplankton, all tissue sampled was utilised to generate the greatest number of replicates possible (n = 1–3). Adequate samples of zooplankton to enable analyses were only collected during trips 2 (sites 1, 4, 5 and 6) and 3 (sites 1 and 5). Sandy sprat and zooplankton tissue samples were dried for 36 hours at 60°C, homogenised to a fine powder with a mortar and pestle, and kept in a desiccator thereafter. Subsamples were loaded into two sets of tin capsules for isotopic analyses (one for C + N and the other for S). Ideal sample weights for C + N (0.75 mg) and for S (2.5 mg) analyses had been determined *a priori* using various weights from a bulk sample of sandy sprat tissue. Whenever possible, zooplankton samples were analysed in duplicate, with one of the duplicates acidified in its tin capsule with a drop of N HCl to remove carbonates (i.e., a potential component of their exoskeleton).

Isotopic ratios for $^{13}C/^{12}C$ and $^{15}N/^{14}N$ (‰) were measured with a Sercon 20-20 Isotope Ratio Mass Spectrometer (IRMS) at the Waite Campus, Adelaide. Isotope ratios for $^{34}S/^{32}S$ were measured by IRMS at University of California-Davis. Isotope ratios were expressed following the δ ('del') convention where, for $^{13}C/^{12}C$ (R):

$$\delta^{13}C\ (‰) = (R_{sample}/R_{standard}) - 1) * 1000$$

Spatio-temporal variability in the isotopic signature of $\delta^{13}C$, $\delta^{15}N$ and $\delta^{34}S$ of sandy sprat tissue was investigated between sites and sampling events. Data did not conform to the assumptions of parametric ANOVA (Analysis of Variance) and, thus statistical tests were undertaken with two-factor (i.e. site and sampling event) univariate PERMANOVA in the software package PRIMER v. 6.1.12 and PERMANOVA+ (Anderson *et al.* 2008). These analyses were undertaken on Euclidean Distance similarity matrices. The B-Y method significance correction was applied when multiple comparisons were undertaken (Benjamini and Yekutieli 2001, Narum 2006).

Isotopic signatures of $\delta^{13}C$, $\delta^{15}N$ and $\delta^{34}S$ from sandy sprat collected from the Murray estuary in the current study, were compared qualitatively with isotopic signatures of sandy sprat collected from the Murray estuary in 2007 (Deegan *et al.* 2010; Lamontagne Unpublished data) and 2013 (Johnson 2014).

Compound-specific stable isotope analyses

Samples of sandy sprat from trip 1 were trialed for CSIA using amino acids to provide supporting information to traditional SIA. Samples were prepared adopting a modified procedure to that of Brand *et al.* (1994) and Hofmann *et al.* (2003) using dried sandy sprat tissue (prepared as above). The $\delta^{15}N$ isotope composition of the amino acids were determined with a Trace GC gas chromatograph interfaced with a Delta V Plus mass spectrometer through a GC-C combustion furnace (1030°C), reduction furnace (650°C) and liquid N_2 cold trap. The samples (0.5 µL) were injected splitless (split/splitless injector, 10:1 split ratio) onto a forte BPX5 capillary column (30 m × 0.32 mm × 1.0 µm film thickness) at an injector temperature of 180°C with a constant helium flow rate of 1.5 ml min^{-1}. The column was initially held at 50°C for 2 min and then increased to 120°C at a rate of 10°C min^{-1}. Once at 120°C, the temperature was increased at a rate of 4°C min^{-1} to 195°C and then at 5°C min^{-1} to 235°C where it was held for 5 min. The temperature was then further increased to 300°C at 15°C min^{-1} and held for 8 minutes. All samples were analysed at least in triplicate.

$\delta^{15}N$ values were normalised as follows. Each sample analysis consisted of three separate IRMS analyses bracketed by a suite of amino acids with known $\delta^{15}N$ values. The slope and intercept of known vs measured values were then used to correct the measured values for the sample set. In addition, an internal reference compound, norleucine, also of known nitrogen isotopic

composition, was co-injected with samples. The norleucine provided a check of combustion conditions and the consistency of normalisation using the bracketing standards. The $\delta^{13}C$ values of individual amino acids were measured as per for $\delta^{15}N$. To correct for added C and isotope fractionation during derivatization, amino acid $\delta^{13}C$ values were corrected based on analysis of pure amino acid standards that were prepared and analyzed under the same conditions as the samples. Reproducibility associated with isotopic analysis of glutamic acid and phenylalanine averaged ±0.44‰ (1 SD) and ranged from ±0.06‰ to ±0.85‰.

The trophic position of each fish species was calculated using the measured $\delta^{15}N$ values of glutamic acid (Glu) and phenylalanine (Phe) as described by Chikaraishi *et al.* (2009) as follows:

$$TP = \frac{\delta^{15}N_{Glu} - \delta^{15}N_{Phe} - 3.4}{7.6} + 1$$

where TP is the trophic position determined, 3.4 is the isotopic difference between glutamic acid and phenylalanine in the primary producers (β) and 7.6 is the assumed trophic enrichment factor (TEF). Trophic position as calculated from CSIA was qualitatively compared with that calculated from traditional SIA.

Due to the novel nature of CSIA and its current limited use in estuaries, results of $\delta^{13}C$ analyses of amino acids (i.e. leucine, isoleucine, lysine, glutamic acid and phenylalanine) of sandy sprat collected from the Murray estuary in 2014 are compared qualitatively with published data on a range of primary producers (Larsen *et al.* 2009 and 2013).

3 Results

3.1 Hydrology and salinity

Hydrology in 2014 was characterised by generally low discharge (total barrage discharge typically <2000 ML.day^{-1}) until August 2014 when flow increased abruptly and remained elevated for a period of ~23 days (Figure 6a). During this period, discharge from both Goolwa and Tauwitchere Barrages peaked at ~11,000 ML.day^{-1}. Discharge had decreased by September 2014 and ranged from 0–1900 ML.day^{-1}, but was predominantly ~1000 ML.day^{-1} at both barrages throughout September and most of October. At the end of October discharge through Goolwa Barrage was reduced and maintained at an average of 422 ± 27 ML.day^{-1} (range 0–708 ML) throughout November and December, whilst discharge through Tauwitchere was maintained at an average of ~1211 ± 51 ML.day^{-1} (range 7–1676 ML) over the same period.

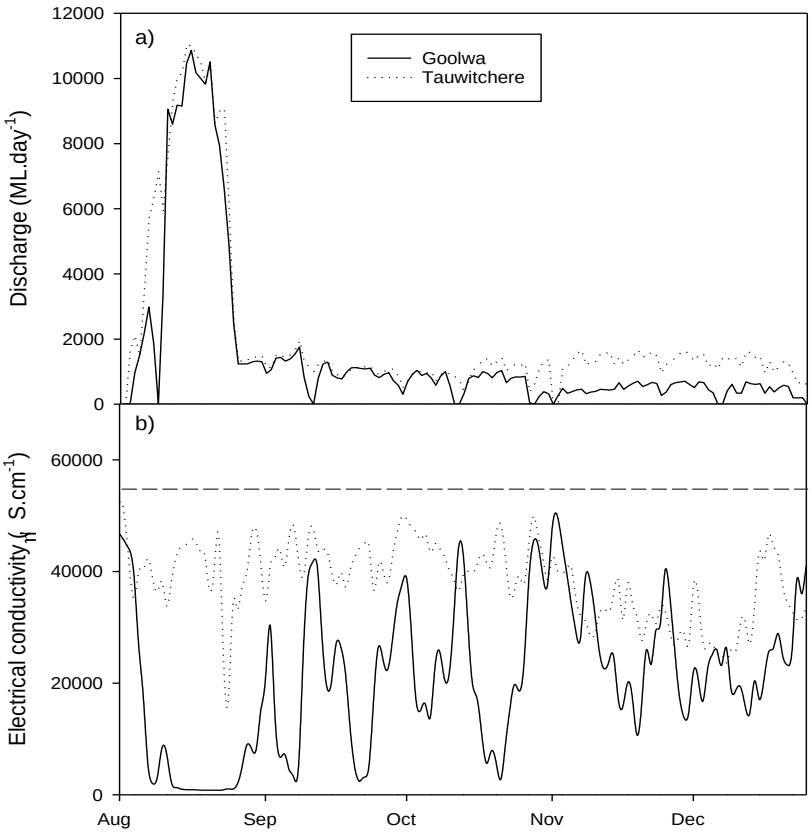

Figure 6. a) Daily discharge (ML.day^{-1}) and b) electrical conductivity (µS.cm^{-1}) downstream of Goolwa (solid line) and Tauwitchere (dotted line) Barrages from 1st August to 31st December 2014. Dashed line represents electrical conductivity of seawater. Sampling trips are indicated by blue shading.

In association with variable discharge, salinity, measured as electrical conductivity (µS.cm^{-1}), was variable downstream of both Goolwa and Tauwitchere Barrages (Figure 6b). Variability was more evident downstream of Goolwa, where the more constrained channel, relative to Tauwitchere, resulted in a marked decrease in conductivity (<1000 µS.cm^{-1}) during higher discharge in August 2014. Conductivity could be considered 'brackish' in the subsequent months, fluctuating regularly through a range 5000–40,000 µS.cm^{-1} during September and October, and 10,000–40,000 µS.cm^{-1} during sampling in November and December 2014. Conductivity downstream of Tauwitchere was also variable, but less so than Goolwa, ranging 24,000–50,000 µS cm^{-1} during sampling.

3.2 Site water quality and phytoplankton community structure

The lowest site-specific conductivities were measured during the first trip (25,000–35,000 µS.cm^{-1}) and conductivity generally increased across trips (Figure 7a). During each trip there was a consistent pattern of lower conductivities near the barrages at Goolwa and Tauwitchere with values increasing with distance from the barrages and peaking at Godfrey's Landing. During the third trip conductivities similar to seawater were measured at Godfrey's Landing, Ewe Island and Tauwitchere (~50,000 µS.cm^{-1}), while conductivities at Goolwa downstream and Rushy Island were ~35,000 µS.cm^{-1}. Patterns of conductivity were reflected by other water quality attributes.

Spatio-temporal variability in TP (Figure 7b) and TKN concentrations (mg.L^{-1}) (Figure 7c) were generally the inverse of conductivity, with concentrations higher nearer to the barrages and decreasing with distance away. TP was particularly high at Goolwa downstream, exceeding the concentration in the freshwater supply at Goolwa upstream, while at Tauwitchere the concentration was less than observed at Goolwa upstream, but higher than at sites further from the barrages. The concentration of TP declined at Godfrey's Landing, Ewe Island and Tauwitchere across trips as conductivity increased. A small part of the increased phosphorus concentrations at the inflow sites were due to increased concentrations of FRP, with Goolwa downstream having the highest concentrations and Tauwitchere having high concentrations during Trips 1 and 2. In both cases, concentrations were higher than at Goolwa upstream (Figure 7d).

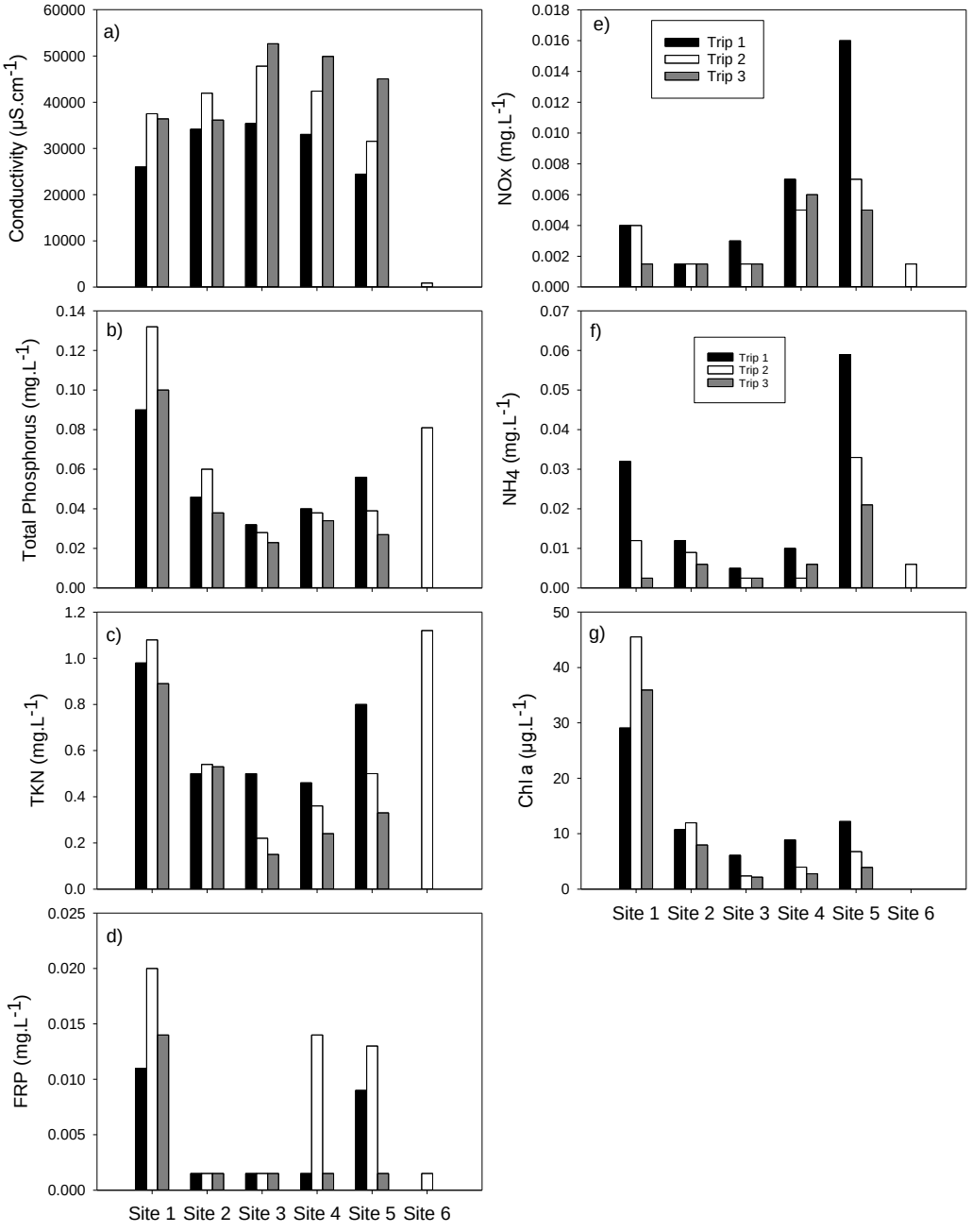

Figure 7. Summary of water quality and nutrient measurements from all sites and sampling trips including a) conductivity (µS.cm^{-1}), b) total phosphorus (mg.L^{-1}), c) TKN (mg.L^{-1}), d) FRP (mg.L^{-1}), e) NOx (mg.L^{-1}), f) NH_4 (mg.L^{-1}) and g) Chl a (µg.L^{-1}). Site 1 = Goolwa downstream, site 2 = Rushy Island, site 3 = Godfrey's Island, site 4 = Ewe Island, site 5 = Tauwitchere, and site 6 = Goolwa upstream.

Concentrations of TKN showed a similar pattern to TP, but concentrations at Goolwa downstream and Tauwitchere were either similar to or less than those at Goolwa upstream. TKN remained consistently high at Goolwa upstream and lower, but constant, at Rushy Island, while at Godfrey's Landing, Ewe Island and Tauwitchere TKN declined across trips as conductivity increased. The

total nitrogen concentration (TN) is the sum of TKN and NO_x, but concentrations of NO_x were low (Figure 7e) so that TN and TKN patterns were similar. TKN measures the concentrations of Total Organic Nitrogen (TON) plus NH_4, but as NH_4 concentrations were very low (Figure 7f), TKN was largely comprised of the dissolved and particulate organic nitrogen compounds, including organisms. TON made up most of the total nitrogen both in the inflowing freshwater and in the estuarine waters. The dissolved inorganic forms of nitrogen, NH_4 and NO_x, showed similar patterns to each other with high concentrations at Goolwa downstream and Tauwitchere exceeding concentrations at Goolwa upstream. Concentrations were particularly high at Tauwitchere and for NO_x high concentrations were also evident at Ewe Island. At Rushy Island and Tauwitchere, both forms of inorganic nitrogen generally declined as salinity increased.

Greater NH_4 concentrations at Tauwitchere and Goolwa downstream, relative to Goolwa upstream, suggest the breakdown of organic materials with high NO_x concentrations and nitrification of the ammonium. This is supported by greater FRP concentrations at Tauwitchere and Goolwa downstream, relative to Goolwa upstream (Figure 7d). Both NO_x and FRP occasionally occurred at high concentrations at Ewe Island suggesting transport from Tauwitchere. This contrasted with Goolwa downstream where concentrations of the dissolved inorganic forms did not reflect substantial transport to Rushy Island, suggesting their rapid removal from the water column. Their rapid removal into particulate form was supported by the patterns of TP and TKN, which suggested transport of total nutrients from Goolwa downstream to Rushy Island (Figure 7a and c).

The distribution of chlorophyll-a, an indicator of phytoplankton biomass, was aligned with nutrient patterns (Figure 7g). Chlorophyll concentrations were high at Goolwa downstream on all occasions, with a particularly high peak during trip 2. Concentrations at Rushy Island were substantially less than those at Goolwa downstream, but were similar to, or greater than those at Tauwitchere, the other freshwater inflow zone. In general the chlorophyll concentrations at Godfrey's Landing, Ewe Island and Tauwitchere were lower than at Goolwa downstream and Rushy Island and declined between trip 1 and 3.

Direct comparison of nutrient types provides further insight on potential biogeochemical linkages. Total organic carbon (TOC) and total organic nitrogen (TON) were significantly correlated (r^2 = 0.855, $p < 0.05$), except for Goolwa downstream with a higher organic nitrogen concentration (Figure 8a). On average the ratio of TOC to TON (~7:1) was typical of phytoplankton and other biota suggesting a productive zone. The chlorophyll-a (Chl a) concentration was strongly correlated with TON (r^2 = 0.808, $p < 0.05$) except for Goolwa

downstream where chlorophyll concentrations were significantly higher (Figure 8b). This indicates a change in the composition of the phytoplankton to more chlorophyll enriched groups.

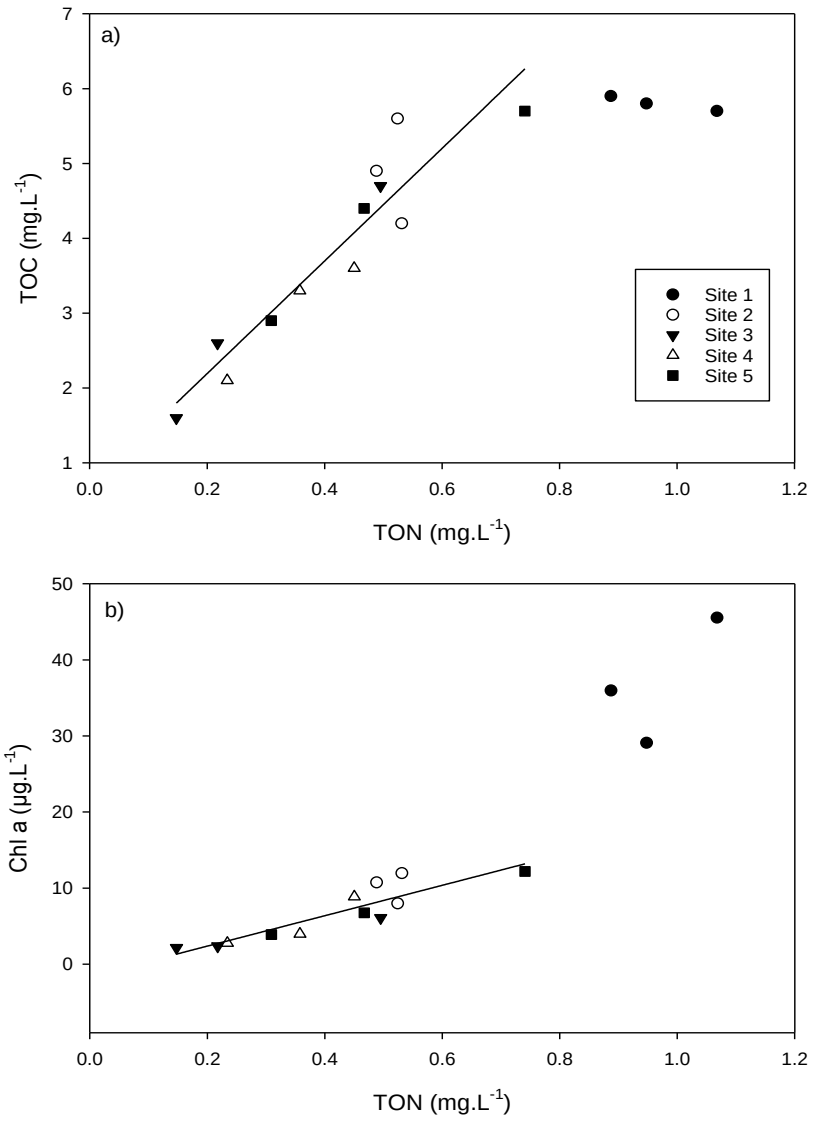

Figure 8. Plots and linear regressions of a) TOC ($mg.L^{-1}$) verse TON ($mg.L^{-1}$) and b) Chl a ($\mu g.L^{-1}$) verse TON ($mg.L^{-1}$) for all sites and trips.

Phytoplankton counts only approximated phytoplankton concentrations determined from chlorophyll analyses, with high numbers occurring at Goolwa downstream and Tauwitchere, but with greater concentrations at Tauwitchere than Goolwa downstream during trips 1 and 2, contrary to chlorophyll results (Figure 9a). Nonetheless, chlorophyll measurements provide an estimate of cell biomass rather than cell number. Also, cyanobacteria have low chlorophyll content and the phytoplankton community was dominated by cyanobacteria (Figure 9b), with this comprised largely of *Aphanocapsa* sp. with a small cell size (1–2 µm diameter).

The different phytoplankton genera were considered to be either marine or freshwater forms based on published information. However, not all could be readily assigned and in some cases the allocations were influenced by the observed lack of particular groups in the lake compared to the estuary. The freshwater phytoplankton carried into the Murray Estuary declined rapidly so concentrations at Goolwa downstream and Tauwitchere were less than those in the lake. Cyanobacteria and chlorophytes showed similar patterns (Figure 9b and c). In general these two groups decreased at each site over time as salinity increased, and decreased across the sites during trips in accord with increasing salinity, with lowest concentrations at Godfrey's Landing. Compared to the concentrations at Goolwa upstream, the reduction in chlorophytes was substantial, while the concentrations of cyanobacteria remained significantly higher than other phytoplankton across all of the sites. Nonetheless, a steady decline in cyanobacteria suggested that they were not growing under these conditions.

Several marine diatom genera were detected in the Murray estuary at low concentrations, but were absent from Goolwa upstream (Figure 9d), whereas diatom genera detected at Goolwa upstream were not observed in the estuary. Dinoflagellates and cryptophytes, which were considered to be marine or estuarine genera as they were not observed at Goolwa upstream, had high concentrations, especially at Goolwa downstream, suggesting that they were growing at this location (Figure 9e and f).

Phytoplankton identified through the molecular analyses included the same groups as those identified using traditional taxonomic methods, Chlorophyta, Euglenophyta, Cryptophyta, Dinophyta and Bacillariophyceae, but in addition included Haptophyta (Prymnesiales). A detailed comparison of the taxonomic identifications at lower levels, and the quantification of the phytoplankton by the different methods were beyond the means of this project.

The changes in phytoplankton community composition across sites and trips, and the influences of water quality attributes are depicted in a MDS ordination based on the average phytoplankton counts at each site and overlayed with the water quality data (Figure 10). A similar pattern is evident for each trip where Goolwa downstream and Tauwitchere are relatively close, with the other sites, especially Godfrey's Landing and Ewe Island, further away. This pattern then progresses across the two-dimensional space with each trip. The overlay of water quality attributes for each site indicates that this progression is aligned with increasing conductivity and reductions in the organic material present and decreases in major nutrients and turbidity.

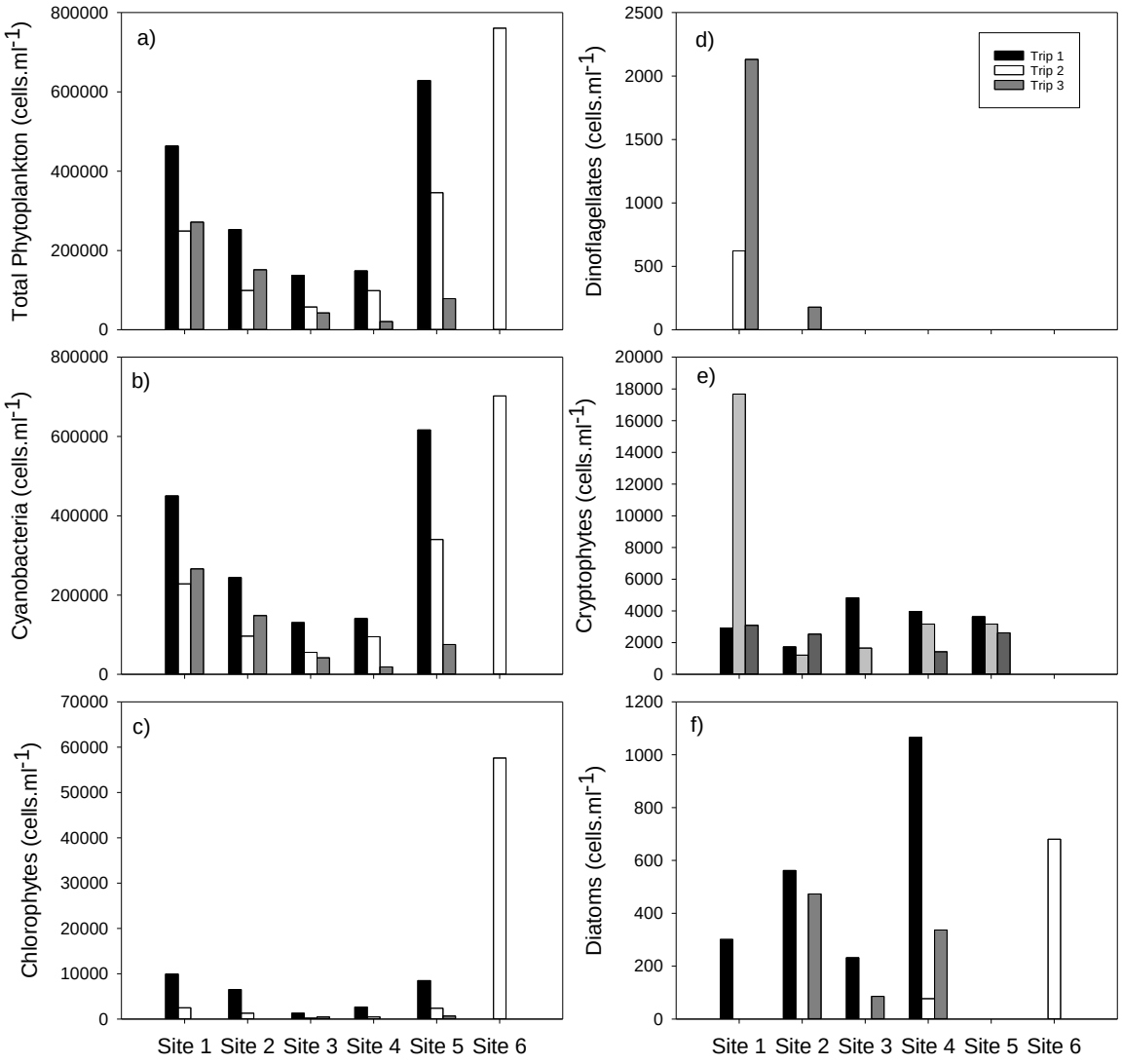

Figure 9. Abundances of phytoplankton from all sites and trips including a) total phytoplankton b) cyanobacteria, c) chlorophytes, d) dinoflagellates, e) cryptophytes and f) diatoms. All data is presented as cells.ml^{-1}. Site 1 = Goolwa downstream, site 2 = Rushy Island, site 3 = Godfrey's Island, site 4 = Ewe Island, site 5 = Tauwitchere, and site 6 = Goolwa upstream.

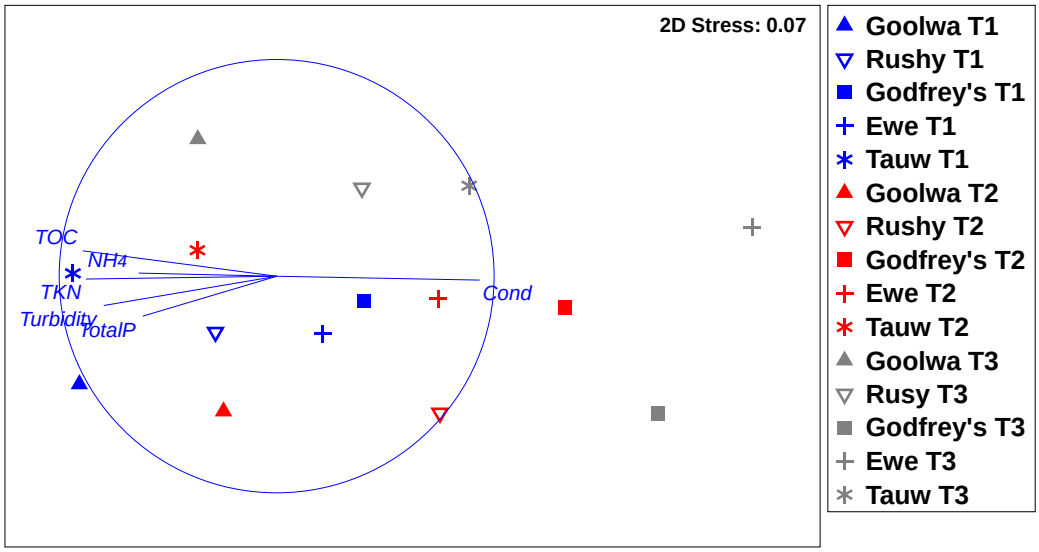

Figure 10. Non-metric Multidimensional Scaling ordination (MDS) of phytoplankton community composition (i.e. species identity and abundance) across trips. Where Goolwa downstream = Goolwa, Rushy Island = Rushy, Godfrey's Landing = Godfrey's, Ewe Island = Ewe, Tauwitchere = Tauw, T1 = trip 1, T2 = trip 2 and T3 = trip 3. Ordination was performed on a Bray-Curtis similarity matrix of untransformed site average count data.

3.3 Microbiota/zooplankton community structure

Quantitative identification and enumeration

In total, 31 taxa were identified from trap samples comprising 22 rotifer and 3 cladoceran species, 3 orders of copepod, ostracods, amphipods and decapods. The average number of species present within trap samples ranged from zero at Rushy Island during trip 1, to 14 at Goolwa upstream during trip 2 (see Appendix 1 for a detailed summary of taxa and relative proportions). Total abundance of zooplankton (all species combined) varied between trips and sites. For sites within the Murray estuary, abundance ranged from 4.25 ± 4.25 ind.L^{-1} at Rushy Island during trip 3, to 292.78 ± 29.42 ind.L^{-1} at Rushy Island during trip 1 (Figure 11). Total zooplankton abundance, however, was greatest (570.83 ± 64.14 ind.L^{-1}) during the single sampling event at Goolwa upstream during trip 2 (this data was excluded from statistical analyses). This abundance measure equates to a potential zooplankton load of 384 ± 241 kg.GL^{-1} transported to the Coorong with freshwater discharge at this time.

PERMANOVA indicated there was a significant interaction between site and trip (*Pseudo-*$F_{8, 44}$ = 10.53, $p < 0.001$) suggesting temporal variability in total abundance was not consistent across sites within the Murray estuary. Pairwise comparisons revealed that mean total abundance did

not change significantly between trips at Godfrey's landing ($p > 0.05$). At both Rushy Island and Ewe Island, significant declines in total abundance were evident across sampling trips ($p < 0.05$). Total abundance at Tauwitchere and Goolwa downstream also varied significantly between trips ($p < 0.05$), as driven by peak abundances during trip 2.

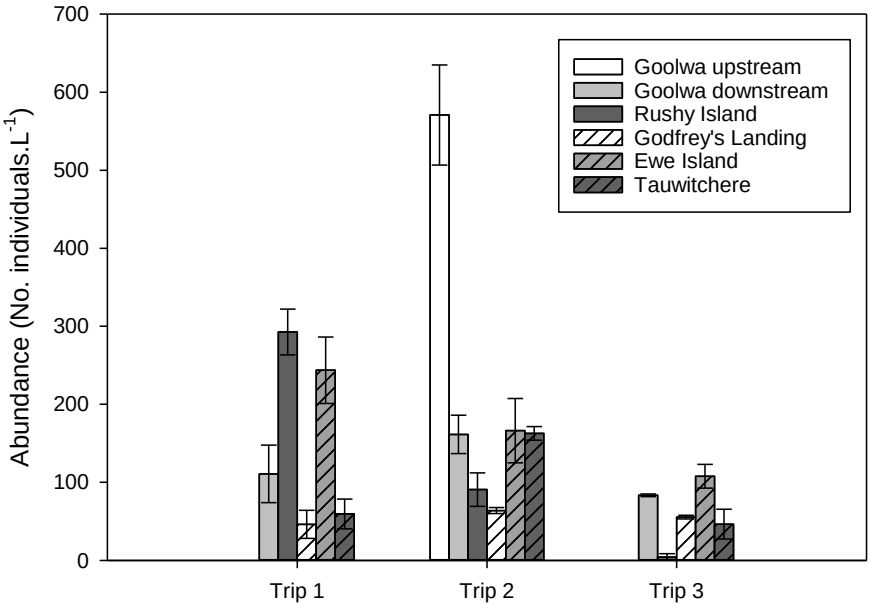

Figure 11. Zooplankton abundance (all species combined; ind. L^{-1} ± SE) for all sites and trips.

During trip 1, the freshwater rotifer species *Keratella australis* and *Filinia australiensis* were abundant at Tauwitchere (12% and 16% of the community by number, respectively) and Goolwa Channel (20% and 4%, respectively) and the freshwater cladoceran species *Bosmina meridionalis* at Tauwitchere (6%). The rotifer *Synchaeta* cf. *triophthalma*, a marine/brackish species, was present at all sites where it made up relatively high proportions of the community (~15% at Rushy Island and Tauwitcherie and ~64% at Ewe Island).

During trip 2 the freshwater rotifer species *Brachionus calyciflorus* and *Brachionus keikoa* were present at both the upstream and downstream Goolwa sites (5–8%). The freshwater rotifer species *K. australis* was present at Tauwitcherie (11%), Goolwa downstream (30%), Goolwa upstream (23%) and Rushy Island (5%). In total 10 taxa were recorded at Goolwa downstream, 7 of which were also recorded at Goolwa upstream. Again *S.* cf. *triophthalma* made up relatively high proportions of the community at Rushy and Ewe Island (42% and 18%, respectively) yet only a small proportion at Goolwa downstream (5%) and was absent at Godfreys Landing and

Tauwitchere. The estuarine rotifer *Synchaeta vorax* made up a relatively high proportion of the community at Godfreys Landing (14%). Prior to this study there has been no official recording of this species within Australian waters.

During trip 3, copepod nauplii (copepod orders not discriminated at the naupliar stage) made up the majority or all of the community at Rushy Island (100%), Tauwitchere (70%) and Goolwa downstream (80%) and a considerable proportion at Ewe Island (28%) and Godfreys Landing (21%). Again *S.* cf. *triophthalma* made up a considerable proportion of the community at Ewe Island (29%) and Godfreys Landing (30%).

MDS ordination of zooplankton community data exhibited interspersion of samples with weak grouping by site and trip (Figure 12). Community structure (species composition and abundance) differed significantly between trips (*Pseudo-*$F_{2, 42}$ = 4.02, $p < 0.001$) and sites (*Pseudo-*$F_{4, 42}$ = 4.85, $p < 0.001$), and there was a significant interaction between trip and site (*Pseudo-*$F_{8, 42}$ = 4.02, $p < 0.001$), indicating that patterns of temporal change in assemblage structure were not consistent across sites. Pairwise comparisons revealed that assemblage structure was significantly different at Goolwa Channel between trip 2 and trip 3, Rushy Island between the trip 1 and trip 2, Godfrey's Landing between the trip 1 and trip 2, and at Tauwitchere, between trip 2 and trip 3 ($p < 0.05$). All other comparisons were non-significant ($p > 0.05$).

Applying a 40% cumulative contribution cut-off, SIMPER indicated the primary contributors to variability between between trip 2 and trip 3 at Goolwa Channel were copepod nauplii, the cladoceran *Bosmina meridionalis* and the rotifers *Filinia longiseta* and *Filinia pejleri*. Excluding copepod nauplii, which increased in abundance, all were abundant during trip 2 and absent or less abundant during trip 3. The primary contributors to variability between trip 1 and trip 2 at Rushy Island were two rotifer species, *Proalides tentaculatus* and *Trichocerca* sp. 1, both of which were abundant during trip 1, but absent during trip 2 (Table 2). Variability between trip 1 and trip 2 at Godfrey's Landing were driven by high abundance of *Synchaeta* cf. *triophthalma* during trip 1 and absence during trip 2 and increased abundance of copepod nauplii between trip 1 and trip 2. Variability between trip 2 and trip 3 at Tauwitchere was primarily driven by declines in the abundance of rotifers *Keratella australis, Trichocerca* cf. *rattus carinata* and *P. tentaculatus* (Table 2).

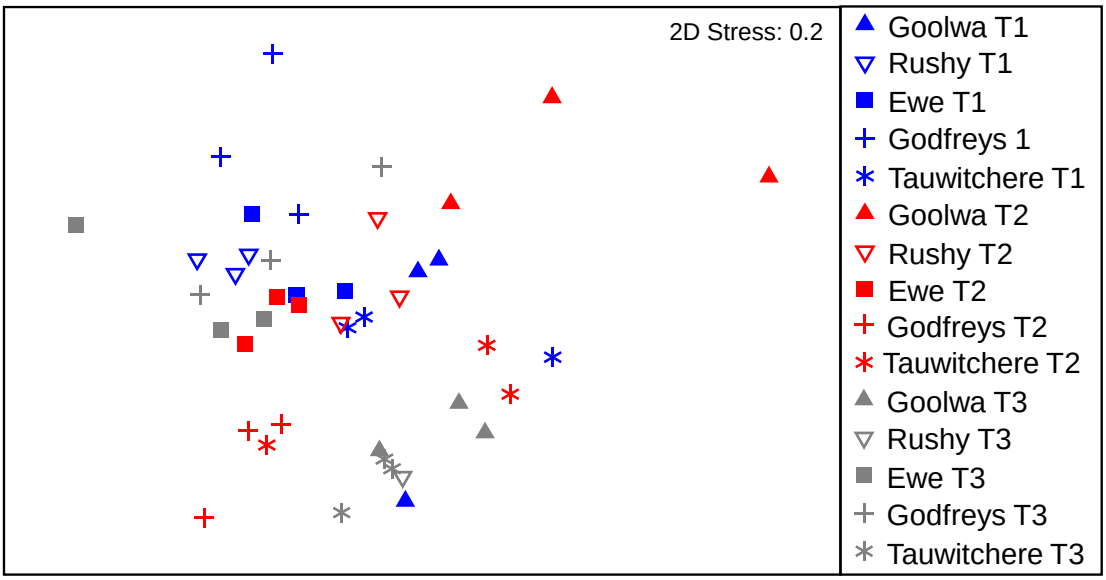

Figure 12. Nonmetric Multidimensional Scaling ordination (MDS) of zooplankton community composition across trips. Where Goolwa downstream = Goolwa, Rushy Island = Rushy, Godfrey's Landing = Godfreys, Ewe Island = Ewe, T1 = trip 1, T2 = trip 2 and T3 = trip 3. Ordination was performed on a Bray-Curtis similarity matrix.

Table 2. Summary of results from SIMPER (Similarity percentages analysis) indicating the species contributing to differences in zooplankton community composition between trip 2 and 3 at Goolwa downstream, trip 1 and trip 2 at Rushy Island, trip 1 and 2 at Godfrey's Landing, and trip 2 and trip 3 at Tauwitchere. A 40% cumulative contribution cut-off was applied.

	Average abundance		Av. Diss	Diss/SD	Contribution	Cumulative contribution
Goolwa	**Trip 2**	**Trip 3**				
All nauplii	0.61	2.85	12.26	2.29	15.22	15.22
B. meridionalis	1.98	0	10.8	7.18	13.41	28.63
F. longiseta	1.57	0	8.52	1.32	10.57	39.2
F. pejleri	1.43	0	7.74	1.28	9.61	48.81
Rushy Island	**Trip 1**	**Trip 2**				
P. tentaculatus	3.11	0	15.99	4.52	29.39	29.39
Trichocerca sp 1	2.65	0	13.62	5	25.04	54.42
Godfreys Landing	**Trip 1**	**Trip 2**				
S. cf. triophthalma	2.11	0	18.3	4.59	23.05	23.05
All nauplii	0.62	2.37	17.17	1.53	21.62	44.67
Tauwitchere	**Trip 2**	**Trip 3**				
K. australis	1.47	0	11.76	1.14	18.59	18.59
T. cf. *rattus carinata*	1.38	0	10.61	1.28	16.77	35.36
P. tentaculatus	1.37	0	9.58	1.32	15.14	50.51

Molecular analyses

Molecular analyses yielded approximately 90,000 sequences, comprising a total of 188 OTU's from filtered samples, 167 from net samples and 162 from trap samples, covering a broad range of organisms. Despite different sets of OTU's being collected by the different sampling methods, patterns of variability between trips appeared similar with a progression across the multi-dimensional space from trip 1 to trip 3, and thus, these data were grouped for further analyses. PERMANOVA, based on a Bray-Curtis similarity matrix, indicated significant differences between trips (*Pseudo-*$F_{2, 44}$ = 5.38, $p < 0.001$), and sites (*Pseudo-*$F_{4, 44}$ = 1.68, $p = 0.019$) with no significant interaction (*Pseudo-*$F_{8, 44}$ = 0.99, $p = 0.488$). Differences between sites were significant (B-Y method corrected $\alpha = 0.017$) for comparisons of Goolwa downstream with both Godfrey's Landing and Ewe Island. This is consistent with the water quality and phytoplankton data, where Goolwa downstream was different from other sites, and especially from Godfrey's Landing and Ewe Island. Significant differences were observed between all sampling trips ($p < 0.01$ for all comparisons; B-Y method corrected $\alpha = 0.027$).

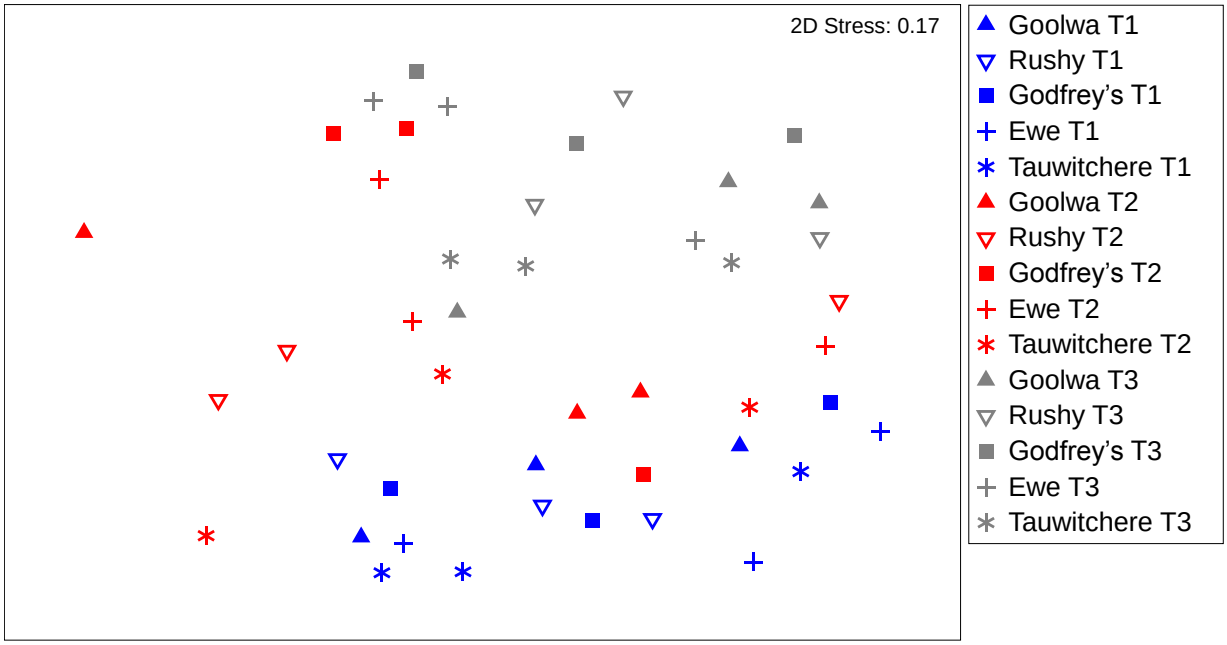

Figure 13. Non-metric Multidimensional Scaling (MDS) ordination of microbiota community composition across trips, based upon proportions of OTU's sampled. Where Goolwa downstream = Goolwa, Rushy Island = Rushy, Godfrey's Landing = Godfreys, Ewe Island = Ewe, T1 = trip 1, T2 = trip 2 and T3 = trip 3. Ordination was performed on a Bray-Curtis similarity matrix.

SIMPER indicated that the abundance of crustaceans played a substantial role in the significant changes in community composition between trips, but with contributions from dinoflagellates, rotifers, bivalves and turbellarian flat worms (Table 3 and Table 4). These samples were aligned with the SILVA database and did not provide sufficient taxonomic resolution to further identify

crustacean groups. This detail was developed further in the analyses of sandy sprat gut content. For simplicity, temporal patterns in community structure are not presented to the detail of individual sites as patterns of change were generally consistent, with decreases in rotifers between trip 1 and trip 2, and increases in crustaceans between trip 2 and trip 3 (Table 3 and Table 4).

Table 3. Summary of results from SIMPER (Similarity percentages analysis) indicating the species contributing to differences in microeukaryote community composition between all trips. A 40% cumulative contribution cut-off was applied.

	Average abundance		**Av. Diss**	**Diss/SD**	**Contribution**	**Cumulative contribution**
All sites	Trip 1	Trip 2				
Euk72	8.43	8.29	6.45	0.96	13.28	13.28
Euk141	0.2	1.51	4.98	0.64	10.24	23.52
Euk84	0.21	1.03	3.61	0.54	7.43	30.95
Euk95	0.77	0.23	2.62	0.64	5.39	36.34
	Trip 1	Trip 3				
Euk72	8.43	9.1	5.59	0.79	13.14	13.14
Euk146	0.08	0.92	2.89	0.65	6.79	19.92
Euk95	0.77	0.27	2.69	0.66	6.31	26.24
Euk94	0.43	0.45	2.31	0.55	5.43	31.67
	Trip 2	Trip 3				
Euk72	8.29	9.1	5.64	0.88	13.12	13.12
Euk141	1.51	0.22	4.9	0.67	11.4	24.51
Euk84	1.03	0.32	3.74	0.58	8.7	33.21
Euk146	0.12	0.92	2.75	0.67	6.41	39.61

Table 4. Key to classification of eukaryote OTU's identified by SIMPER as substantially contributing to differences in microeukaryote community composition between sampling trips.

OTU	**Classification**	**Classification**
Euk72	Arthropoda	Crustacea
Euk141	Gymnodinium clade	Dinoflagellata
Euk84	Mollusca	Bivalvia
Euk95		Rotifera
Euk146	Suessiaceae	Dinoflagellata
Euk94	Platyhelminthes	Turbellaria

3.4 Sandy sprat abundance

In 2014/15, sandy sprat was abundant at both Tauwitchere and Goolwa downstream, relative to the preceding eight years (Figure 14). Annual abundance downstream Tauwitchere Barrage was significantly different between years (*Pseudo-*$F_{7, 53}$ = 11.61, $p < 0.001$), but not downstream of Goolwa Barrage (*Pseudo-*$F_{5, 33}$ = 1.25, $p = 0.313$). At Tauwitchere, pairwise comparisons revealed

differences were primarily due to higher abundance during low freshwater discharge in 2006/07, relative to the period of no freshwater discharge in 2007–2010, and further elevated abundances in all years post the high discharge of 2010–2012 (all comparisons $p < 0.013$; B-Y corrected $\alpha = 0.013$). Relative abundance in all years from 2010–2015 was generally similar ($p > 0.013$), but greatest during 2011/12. Abundance was typically greater at Tauwitchere than Goolwa, with the exception of 2009/10.

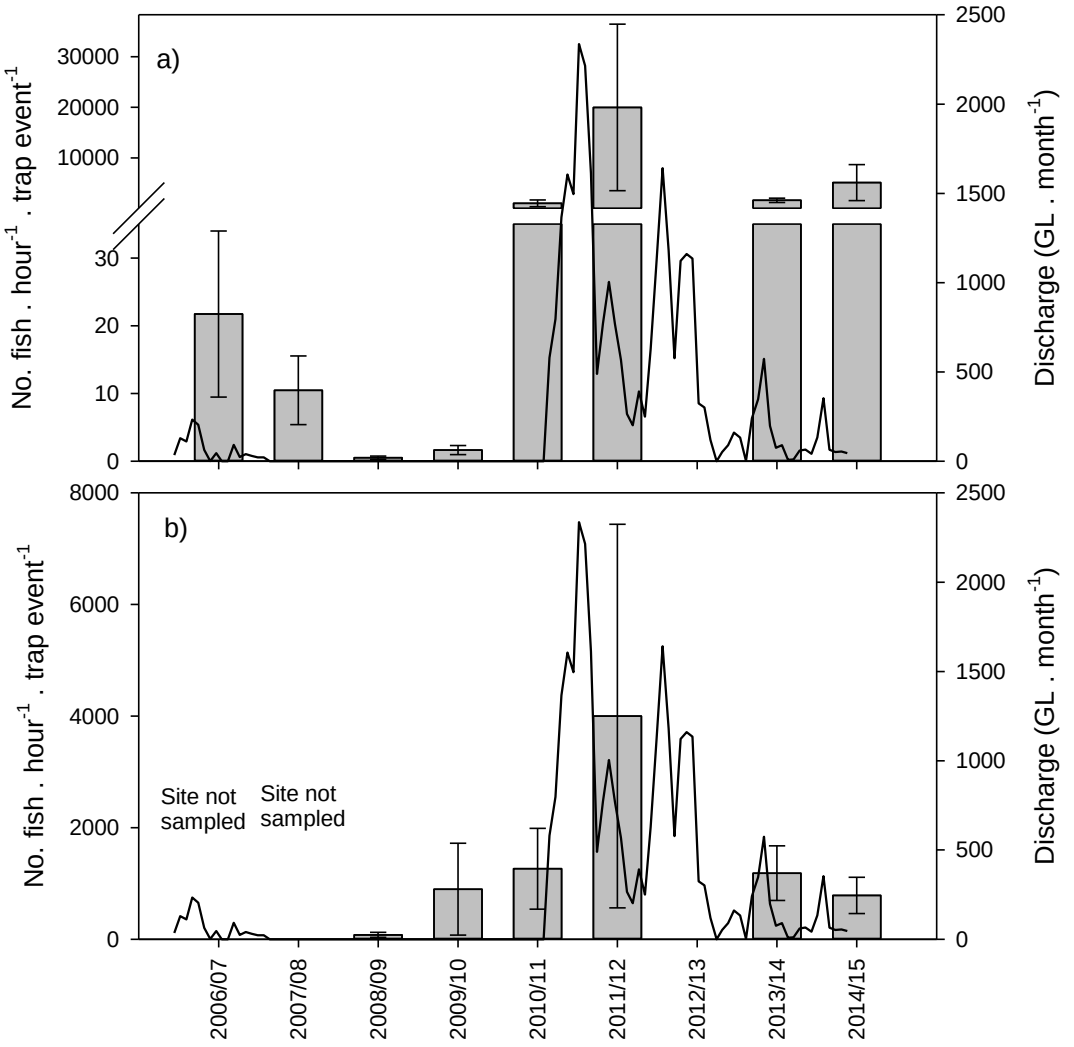

Figure 14. Annual relative abundance (no of fish.hour^{-1}.trap event^{-1}) of sandy sprat sampled at the sites a) downstream Tauwitchere Barrage (site 5) and b) downstream Goolwa Barrage (site 1). Total barrage discharge (GL.month^{-1}) is overlaid on each plot.

Intra-annual variability in the abundance of sandy sprat in 2014/15 was similar between project 1 and 2 (Figure 15). Abundance was typically higher in October and November, and least during December and onwards. Furthermore, abundance was substantially higher downstream of Tauwitchere Barrage than downstream of Goolwa Barrage in November and December.

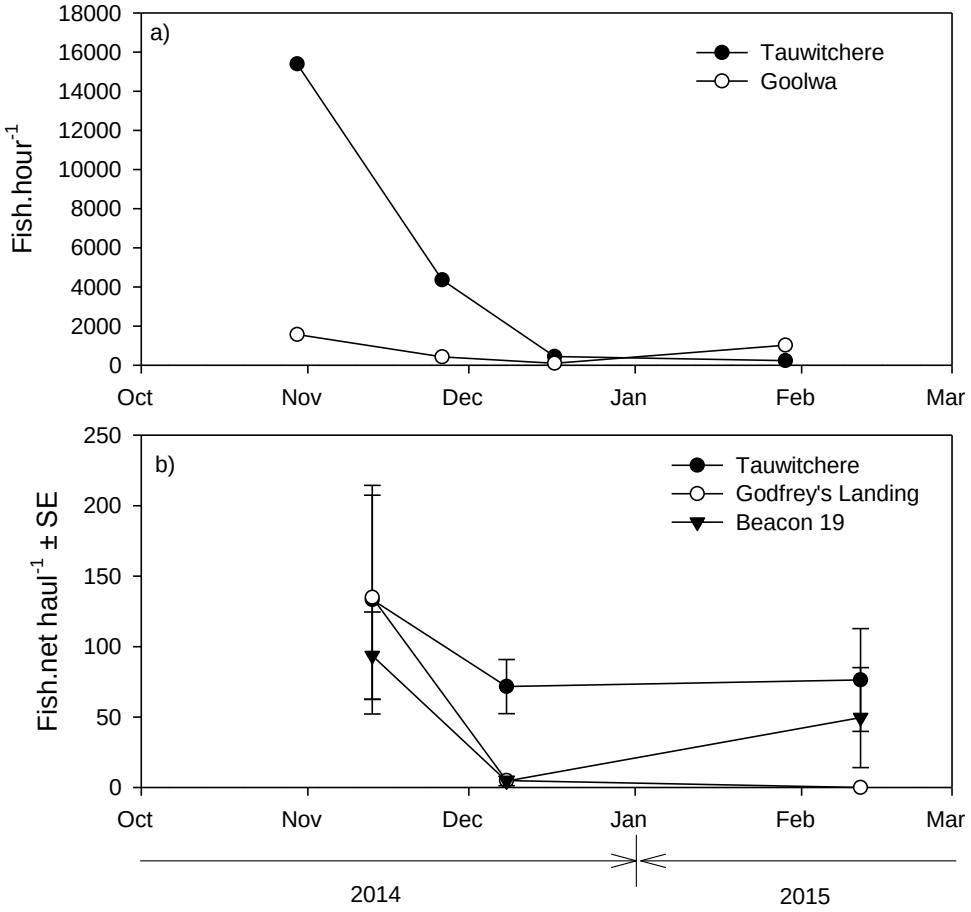

Figure 15. Relative abundance of sandy sprat sampled from sites in the Murray estuary in 2014/15. Data presented include a) relative abundance (fish.hour^{-1}) of sandy sprat sampled from sites downstream Tauwitchere Barrage (site 5) and downstream Goolwa Barrage (site 1), using large double-winged fyke nets and b) relative abundance of sandy sprat (fish.net haul^{-1}) sampled from sites downstream Tauwitchere Barrage (site 5), Godfrey's landing (site 3) and at Beacon 19 (a site situated between sites 1 and 2 from the current study). The blue shaded bars represent the timing of sampling events for the current study, in relation to relative abundance data collected by the above studies.

For sandy sprat captured during sample collection, length distributions (all sites pooled) were similar between trips, with the sampled population ranging 27–43, 25–52 and 23–57 mm FL during trips 1, 2 and 3 respectively (Figure 16). Distribution modes were centred around 30–35 mm FL and individuals <50 mm FL, representing reproductively immature fish (Rogers and Ward 2007), comprised >95% of the sampled population during all trips.

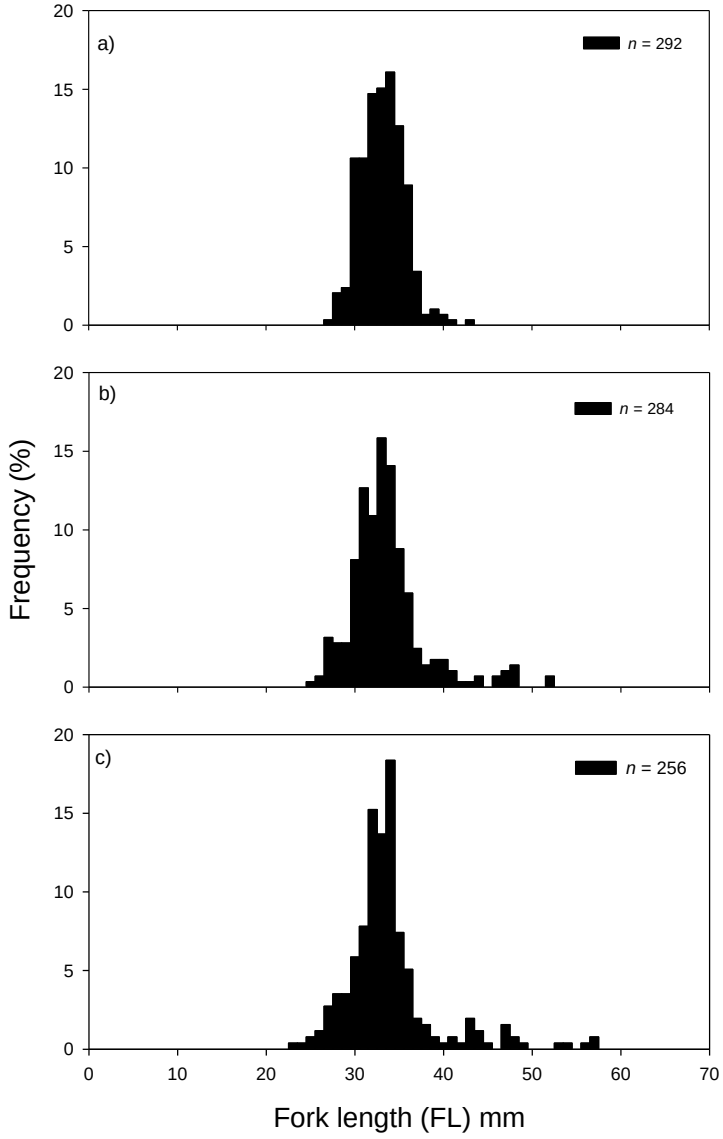

Figure 16. Length-frequency distribution plots for sandy sprat sampled across all sites in the Murray estuary during a) trip 1, b) trip 2 and c) trip 3.

3.5 Sandy sprat diet

Quantitative identification and enumeration

A total of 14 different taxa were identified from sandy sprat guts, with harpacticoid copepods the dominant taxa, comprising ~73% of all prey items. The freshwater cladoceran *Bosmina meridionalis* was the next most abundant prey item (~13%), followed by copepod nauplii and the freshwater rotifer *Keratella australis* (both ~3%). The remaining 10 taxa collectively comprised <8% of total prey items.

The mean number of prey items (ind.fish^{-1} ± SE) at all sites varied between trips, ranging from a minimum of 3.8 ± 1.5 ind.fish^{-1} at Tauwitchere during trip 3, to a maximum of 283 ± 212.8 ind.fish^{-1} at Ewe Island during trip 2 (Figure 17). The mean number of prey items was greatest at Ewe Island (trip 2) and Goolwa downstream (trip 1 and trip 3), and lowest at Rushy Island, Godfrey's Landing and Tauwitchere (Figure 17). A significant interaction between site and trip was detected by PERMANOVA (*Pseudo-*$F_{5, 59}$ = 10.16, $p < 0.001$) indicating temporal variability was not consistent across sites. The absence of fish for gut content analyses from Godfrey's Landing during trips 2 and 3 meant that statistical analysis was not possible for this site. At Ewe Island, total abundance was significantly greater during trip 2 than trip 3 (p = 0.005; B-Y corrected α = 0.017), and at Tauwitchere abundance during trip 1 was significantly greater than trip 2 (p = 0.016), whilst all other comparisons were non-significant ($p > 0.016$).

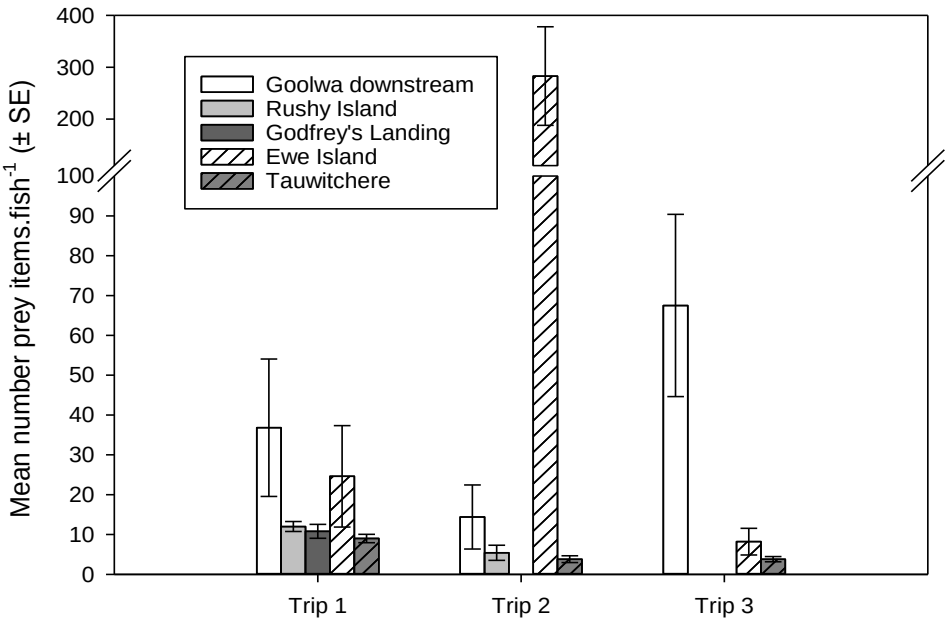

Figure 17. Mean number of prey items (all species combined) from fish sampled at all sites during trips 1, 2 and 3.

MDS ordination of fish gut content data exhibited groupings of samples by trip and site (Figure 18). This was supported by PERMANOVA, which indicated that gut content (species composition and abundance) differed significantly between trips (*Pseudo-*$F_{2, 58}$ = 12.65, $p < 0.001$) and sites (*Pseudo-*$F_{4, 58}$ = 10.90, $p < 0.001$), and there was a significant interaction between trip and site (*Pseudo-*$F_{5, 58}$ = 8.28, $p < 0.001$), suggesting temporal variability in gut content was not consistent across sites. Pairwise comparisons revealed significant differences in sandy sprat diet at Rushy Island between trip 1 and trip 2 (p = 0.013; B-Y corrected α = 0.017), Ewe Island between all trips,

Tauwitchere between trip 2 and trip 3 ($p \leq 0.008$ for all comparisons), and Goolwa downstream between trip 1 and trip 3 ($p = 0.006$).

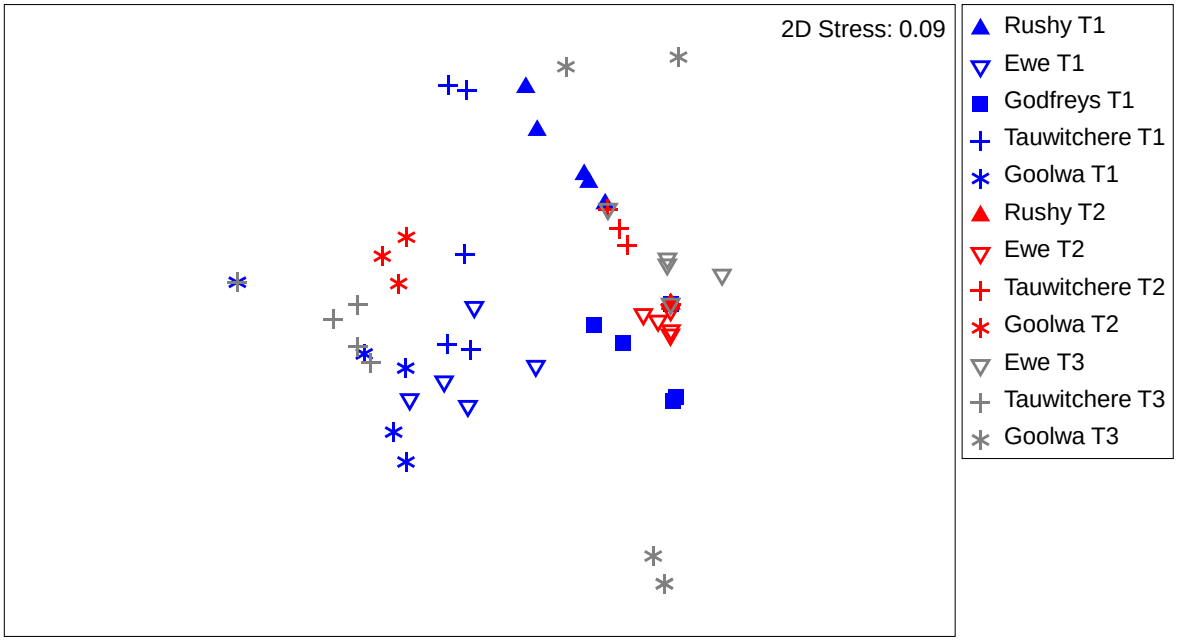

Figure 18. Nonmetric Multidimensional Scaling ordination (MDS) of sandy sprat gut content (i.e. species identity and proportion) across trips and sites. Where Goolwa downstream = Goolwa, Rushy Island = Rushy, Godfrey's Landing = Godfreys, Ewe Island = Ewe, T1 = trip 1, T2 = trip 2 and T3 = trip 3. Ordination was performed on a Bray-Curtis similarity matrix.

The freshwater cladoceran *B. meridionalis* comprised >70% of the gut content of sandy sprat during trip 1 at both Ewe Island and Goolwa downstream (Figure 20). Declines in abundance of this species within sandy sprat guts or complete absence, contributed greatly to variability in sandy sprat diet between trip 1 and subsequent trips, as indicated by SIMPER (Table 5). Increases in the abundance of harpacticoid copepods and copepod nauplii (all species) across trips also contributed to temporal variability at these sites. Harpacticoid copepods constituted >80% of dietary items at Ewe Island during both trips 2 and 3. Increases in harpacticoid copepods also drove differences in diet between trips 1 and 2 at Rushy Island, together with decreases in the abundance of amphipods. In contrast, differences in diet between trips 2 and 3 at Tauwitchere were primarily driven by decreased abundance of harpacticoid copepods and increased abundance of the rotifer *Keratella australis.*

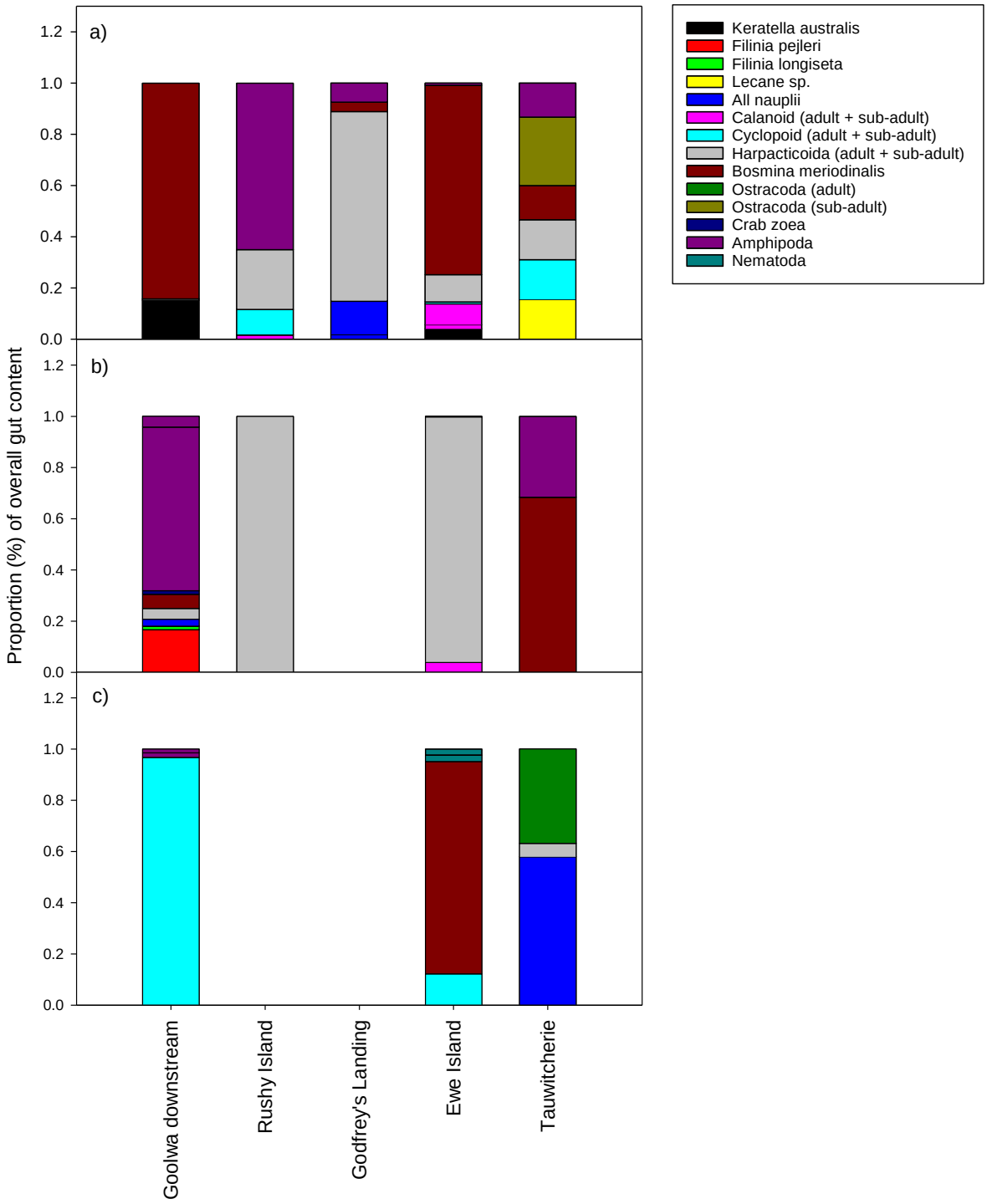

Figure 19. Individual food types expressed as an average percentage of the total number of food items found in the stomach at each site during a) trip 1, b) trip 2 and c) trip 3.

Table 5. SIMPER analysis results table indicating the proportion of variability in sandy sprat diet between trip 1 and trip 2 at Rushy Island, all trips at Ewe Island, trip 2 and 3 at Tauwitchere and trip 1 and 3 at Goolwa Barrage associated with individual prey types.

	Average abundance		Av. Diss	Diss/SD	Contribution	Cumulative contribution
Goolwa downstream	Trip 1	Trip 3				
Bosmina meridionalis	4.16	0	31.09	1.81	31.09	31.09
All nauplii	0	3.42	26.43	1.19	26.43	57.52
Rushy Island	Trip 1	Trip 2				
Amphipoda	4.57	0	31.87	6.4	48.03	48.03
Harpacticoida	2.47	5.74	23.07	2	34.77	82.81
Ewe Island	Trip 1	Trip 2				
Bosmina meridionalis	4.56	0.06	26.72	5.53	36.66	36.66
Harpacticoida	1.83	5.56	22.14	3.15	30.38	67.03
	Trip 1	Trip 3				
Bosmina meridionalis	4.56	0	26.63	5.47	34.81	34.81
Harpacticoida	1.83	4.99	18.61	2.30	24.33	59.14
	Trip 2	Trip 3				
All nauplii	1.27	0	8.74	2.42	28.59	28.59
Cyclopoid	0	0.93	6.36	0.8	20.81	49.40
Amhipoda	0.16	0.81	6.01	0.59	19.66	69.06
Tauwitchere	Trip 2	Trip 3				
Harpacticoida	4.86	0	32.13	4.9	32.13	32.13
Keratella australis	0	4.43	29.44	3.5	29.44	61.57

The Strauss index provides a means of determining the selectivity of sandy sprat for particular prey items by integrating data on ambient prey availability and the prevalence of particular prey items in gut contents. In association with the high prevalence of *B. meridionalis* in gut content samples from Goolwa downstream and Ewe Island during trip 1, and from Tauwitchere during trip 3, sandy sprat exhibited high Strauss index values and were selectively predating upon *B. meridionalis* (Figure 20). At Rushy Island and Godfrey's Landing during trip 1, sandy sprat exhibited the greatest selectivity for amphipods and harpacticoid copepods, respectively. Harpacticoid copepods were also selectively predated at Tauwitchere, Ewe Island and Rushy Island during trip 2, whilst crab zoea were selectively predated at Goolwa downstream. Harpacticoid copepods remained the taxa most selectively preyed upon at Ewe Island during trip 3, whilst the rotifer *K.australis* and copepod nauplii were selectively preyed upon at Tauwitchere and Goolwa downstream, respectively (Figure 20). Indeed, copepod nauplii comprised ~97% of items in the gut content at Goolwa downstream during trip 3, but in contrast, were typically selected against at other sites and during previous trips. The rotifers *Synchaeta triophthalma* and *P. tentaculatus* were consistently selected against by sandy sprat (Figure 20).

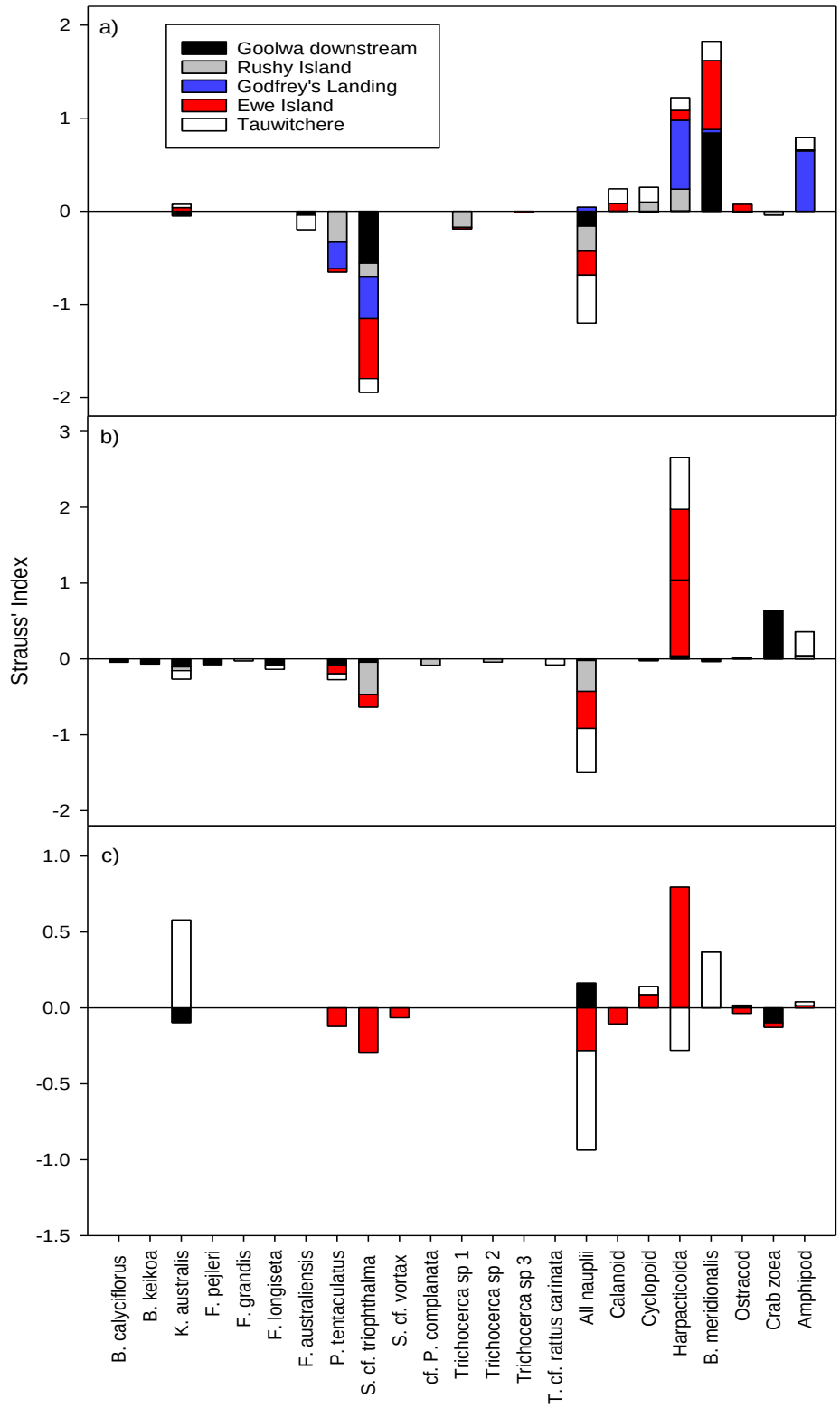

Figure 20. Strauss index of food selectivity of sandy sprat during a) trip 1, b) trip 2 and c) trip 3. Possible values range from +1, which indicates perfect selection for a prey type, and –1, which indicates perfect selection against a prey type. The Strauss index for food selectivity was calculated for each individual prey type at each site for each trip. Values from each site are summed for each trip. No fish were available for gut content analyses from Godfrey's Landing from trips 2 and 3, or Rushy Island from trip 3.

Molecular analyses

A total of 100 OTU's were identified from gut content samples when aligned with the SILVA database. Of these 17 were not found in filtered, trap or net samples (see Section 3.2), but were associated with parasites, fungi and other 'non-prey' gut content. The eight most prevalent OTU's in the gut contents were considered major contributors to diet, contributing ≥1% to the average OTU total in the fish gut samples, and generally, but not always occurred at higher concentrations in the gut content than in the field samples (Table 6). It was evident from this analysis that crustaceans formed the major component of the diet of the Sandy Sprat. It was not possible to further classify the crustaceans into lower taxonomic groupings using the SILVA database and so the crustacean sequences, along with a number of unassigned sequences were aligned with the GenBank database. This provided an improved understanding of the OTU's contributing to the gut content.

Table 6. Proportion of OTU's detected from filtered water samples, zooplankton net and trap samples, and sandy sprat gut content samples. These results reflect alignment of sampled sequences with the SILVA database only.

Organism	Filter samples	Net samples	Trap samples	Gut samples
Crustacea	8.98	14.27	25.34	76.85
Dinoflagellate	0.36	0.33	0.22	3.71
Bivalvia	1.59	2.16	5.05	2.42
Turbellaria	0.19	0.00	0.04	1.55
Rotifera	2.60	2.97	2.85	1.15
Dinoflagellate	0.64	0.55	0.53	1.04
Appendicularia	0.56	0.81	0.77	1.03
Dinoflagellate	0.36	0.33	0.22	3.71

Whilst generally informative, use of the GenBank database resulted in both unassigned and poorly identified sequences and these included organisms that significantly influenced the fish gut content (eg. OTUs 6 and 8, Table 7). Consequently, caution must be exercised when interpreting diet, especially the determination of the role of freshwater organisms, as unassigned OTU's could not be attributed to marine or non-marine groups. Nevertheless, comparison of gut content OTU's between trips and sites, using PERMANOVA, showed there was a significant interaction between trips and sites ($Pseudo\text{-}F_{7,\ 138} = 5.22$, $p < 0.001$) suggesting that temporal

variability in gut content was not consistent among sites. Investigation of temporal variability at individual sites showed that sandy sprat gut content was significantly different between all trips ($p \leq 0.003$ for all comparisons) except for Goolwa downstream where gut content was not significantly different between trip 2 and trip 3 ($p > 0.040$; B-Y corrected $\alpha = 0.017$).

SIMPER was used to determine the relative contributions of different OTU's to differences in fish gut OTU composition between trips. To reduce the complexity of the analyses only OTU's contributing >2.5% to the total gut abundance at any site were selected and this resulted in a list of 25 OTU's. These organisms were the major food resources and in general cumulatively contributed between 52 and 84% of the total abundance of OTU's within the gut at any site.

At Goolwa downstream, the major contributors to gut contents were copepod (OTU 2), amphipod (OTU 3 and 4), ostracod (OTU 7), an unassigned crustacean Maxillopoda (OTU 8) and an unassigned taxon (OTU 6) (Table 7). As the identities of OTU 6 and OTU 8 were unresolved, the sources of the food items could not be fully determined. Differences between trips were driven by declining importance of benthic marine/brackish amphipods and increasing importance of ostracods. The freshwater cladoceran *Bosmina* (OTU 30) also contributed >2.5% to gut content during trip 2.

At Tauwitchere during trip 1 there was a larger range of OTU's in the fish gut than observed at other sites. In particular, an unassigned crustacean (OTU 8) and arthropod (OTU 672), amphipod (OTU 3), polychaete (OTU 12), and copepod (OTU 2) made major contributions along with the two freshwater daphniids (OTU's 18 and 24). Amphipod (OTU 3) and copepod (OTU 2) increased in importance during trip 2 and previously absent taxa were detected, including harpacticoid copepod (OTU 5) and an unassigned crustacean (OTU 15) and taxa (OTU 6). During trip 3 the most significant contributors to the gut content were an unassigned arthropod (OTU 672), unassigned crustacean (OTU 8) and amphipod (OTU 3).

At Ewe Island, the organisms observed in gut content were similar to Goolwa downstream, but amphipod (OTU 4) was absent, whilst harpacticoid copepod (OTU 5) was present, and patterns of temporal variability were different between the two sites. During trip 1 the marine copepod (OTU 2) and amphipod (OTU 3), and an unassigned crustacean (OTU 8) and taxa (OTU 6) were dominant along with a freshwater daphniid (OTU 18), shrimp (OTU 26) and cladoceran genus *Bosmina* (OTU 30). During trip 2, two marine copepods (OTU 2 and 5) were dominant almost to the exclusion of other food resources. During trip 3, diet had diversified again with two marine copepods (OTU 2 and 5) and an unassigned taxon (OTU 6) occurring together with a newly appearing cyclopoid copepod (OTU 14) and ostracod (OTU 7). Subsequent to trip 1, all the major contributors to the gut content were marine organisms.

Sandy sprat was collected for molecular analysis of gut content from Godfrey's Landing only during trips 1 and 2. During trip 1, gut content of fish collected from this site was dominated by copepod (OTU 2) and during trip 2 by harpacticoid copepod (OTU 5), calanoid copepod (OTU 20) and an unassigned taxon (OTU 13). The fish gut content at this site reflected largely pelagic food resources with reduced contributions from benthic organisms.

Fish gut contents at Rushy Island appeared to move progressively from a food resource dominated by amphipod (OTU 4) during trip 1 to dominance by copepod (OTU 2) during Trip 2, and dominance by a combination of two copepod taxa (OTU's 2 and 9) during Trip 3.

Table 7. Key to classification of eukaryote OTU's identified by SIMPER as substantially contributing to differences in diet of sandy sprat between sampling trips. Details include OTU number, name, the taxon as defined by the SILVA and GenBank databases, and putative classification of taxa by habitat use i.e. 'marine', 'brackish' or 'freshwater'.

OTU	Name	Taxon	Habitat
2	Copepod	Crustacea; Maxillopoda; Copepoda; Neocopepoda; Podoplea; Misophriodia; Misophriidae; Misophriopsis	Marine
3	Amphipod	Crustacea; Malacostraca; Eumalacostraca; Peracarida; Amphipoda; Gammaridea; Corophiodea; Corophiidae; Corophium	Marine
4	Amphipod	Crustacea; Malacostraca; Eumalacostraca; Peracarida; Amphipoda; Gammaridea; Corophiodea; Aoridae; Aoroides	Marine
5	Hapacticoid copepod	Crustacea; Maxillopoda; Copepoda; Neocopepoda; Podoplea; Harpacticoida; Harpacticidae; Zausodes	Marine
6	Unassigned	Unassigned	Unknown
7	Ostracod	Crustacea; Ostracoda; Podocopa; Podocopida; Cytherocopina; Cytheroidea; Leptocytheridae; Leptocythere	Marine
8	Cruastacea maxillopoda	Unassigned Crustacea; Maxillopoda	Freshwater
9	Copepod	Crustacea; Maxillopoda; Copepoda; Neocopepoda; Podoplea; Misophriodia; Misophriidae; Misophriopsis	Marine
12	Polychaete	Annelida; Polychaeta; Palpata; Aciculata; Phyllodocida; Nereididae; Pseudonereis	Marine
13	Unassigned	Unassigned	Freshwater
14	Cyclopoid copepod	Crustacea; Maxillopoda; Copepoda; Neocopepoda; Podoplea; Cyclopoida; Cyclopidae; Halicyclops	Marine
15	Cruastacea maxillopoda	Unassigned Crustacea; Maxillopoda	Unknown
18	Cladocera daphniidae	Crustacea; Branchiopoda; Phyllopoda; Diplostraca; Cladocera; Anomopoda; Daphniidae; Scapholeberis	Freshwater
19	Cyclopoid copepod	Crustacea; Maxillopoda; Copepoda; Neocopepoda; Podoplea; Cyclopoida; Cyclopidae; Halicyclops	Marine
20	Calanoid copepod	Crustacea; Maxillopoda; Copepoda; Neocopepoda; Gymnoplea; Calanoida; Paracalanidae; Paracalanus	Marine
24	Cladocera daphniidae	Crustacea; Branchiopoda; Phyllopoda; Diplostraca; Cladocera; Anomopoda; Daphniidae; Daphnia	Freshwater
26	Fairy shrimp	Crustacea; Branchiopoda; Sarsostraca; Anostraca; Branchiopodidae; Tanymastigites	Freshwater
30	Bosmina	Crustacea; Branchiopoda; Phyllopoda; Diplostraca; Cladocera; Anomopoda; Bosminidae; Bosmina	Freshwater
38	Flatworm	Platyhelminthes; Rhabditophora; Rhabdocoela; Dalyellioidea; Provorticidae; Pogaina	Brackish
41	Cyclopoid copepod	Crustacea; Maxillopoda; Copepoda; Neocopepoda; Podoplea; Cyclopoida; Oithonidae; Oithona; Oithona sp. 1 New Caledonia-RJH-2001	Marine
49	Green midge	Pancrustacea; Hexapoda; Insecta; Dicondylia; Pterygota; Neoptera; Endopterygota; Diptera; Nematocera; Culicomorpha; Chironomoidea; Chironomidae; Chironominae; Tanytarsini; Tanytarsus	Unknown
59	Shrimp	Crustacea; Malacostraca; Eumalacostraca; Eucarida; Decapoda; Pleocyemata; Caridea; Alpheoidea; Ogyrididae; Ogyrides	Marine
81	Fungi	Eukaryota; Opisthokonta; Fungi	Unknown
470	Amphipod	Crustacea; Malacostraca; Eumalacostraca; Peracarida; Amphipoda; Gammaridea; Corophiodea; Aoridae; Aoroides	Marine
672	Unassigned arthropod	Unassigned Arthropoda	Unknown

3.6 Trophic subsidy

Stable isotope analyses

Adequate numbers of sandy sprat were collected to produce 5 replicate tissue samples for SIA from all sites, during all sampling events, with the exception of Godfrey's Landing during trip 1 (*n* = 3) and Encounter Bay. Several locations within Encounter Bay were sampled during trip 3, but no sandy sprat were collected. Fish sampled and utilised to produce replicate samples were typically juveniles (<40 mm FL), but length was sometimes variable between samples and sites, with low numbers of larger fish (>50 mm FL) sampled. Thus, the number of fish used to produce replicate samples ranged 3–27, but was typically >10. No zooplankton was collected for SIA during trip 1.

Carbon

Acidification decreased the $\delta^{13}C$ of all zooplankton samples from sites within the Murray estuary, but not at Goolwa upstream (Table 8). As not all Coorong zooplankton samples had an acidified duplicate, a standardised $\delta^{13}C$ value was estimated by adding a correction factor (–1.5 ‰) to unacidified samples (expect for samples from upstream Goolwa Barrage).

Table 8. Comparison of $\delta^{13}C$ from acidified and non-acidified zooplankton samples collected during Trip 2 and 3. The standardised $\delta^{13}C$ is either the acidified $\delta^{13}C$ for a sample (when available) or the non-acidified $\delta^{13}C$ with a correction of –1.5‰ (the average difference between non-acidified and acidified replicates); except for the samples from Goolwa upstream where acidification had no effect.

Trip/Sample	$\delta^{13}C$–Non-acidified (‰)	$\delta^{13}C$–Acidified (‰)	Difference (‰)	$\delta^{13}C$–Standardised (‰)
Trip 2				
Goolwa downstream	–20.4	–21.5	–1.1	–21.5
Ewe Island	–18.9	–20.2	–1.3	–20.2
Tauwitchere	–20.1	–	–	–21.6
Goolwa upstream rep 1	–22.3	–22.3	0.0	–22.3
Goolwa upstream rep 2	–22.2	–22.3	–0.1	–22.3
Goolwa upstream rep 3	–22.1	–	–	–22.1
Trip 3				
Goolwa downstream rep 1	–18.8	–20.5	–1.7	–20.5
Goolwa downstream rep 2	–18.7	–20.6	–1.9	–20.6
Goolwa downstream rep 3	–18.9	–20.2	–1.3	–20.2
Tauwitchere	–19.3	–	–	–20.8

Mean $\delta^{13}C$ for sandy sprat collected from sites in the Murray estuary ranged from –20.7‰ at Rushy Island during trip 1, to –18.9‰ at Rushy Island during trip 3. These values are slightly more enriched than mean values for zooplankton from the Murray estuary (–21.6 to –20.1‰) and the mean for zooplankton from Goolwa upstream during trip 2 (–22.3‰; Figure 21). PERMANOVA showed a significant difference in $\delta^{13}C$ between trips (*Pseudo-*$F_{2, 72}$ = 36.13, p < 0.001) and between sites (*Pseudo-*$F_{4, 72}$ = 3.64, p = 0.008), and a significant interaction between trip and site was evident (*Pseudo-*$F_{8, 72}$ = 4.49, p = 0.001). This suggests that values of $\delta^{13}C$ changed between sampling events, but that change was not consistent across sites. Interrogation of mean values of $\delta^{13}C$ between trips for each site indicated $\delta^{13}C$ was not significantly different between trips for fish collected from Godfrey's Landing (p > 0.30 for all comparisons; B-Y corrected α = 0.015), but all other sites exhibited a clear and significant (p < 0.015 for all comparisons) trend of $\delta^{13}C$ enrichment (that is, less negative values) over time (Figure 22).

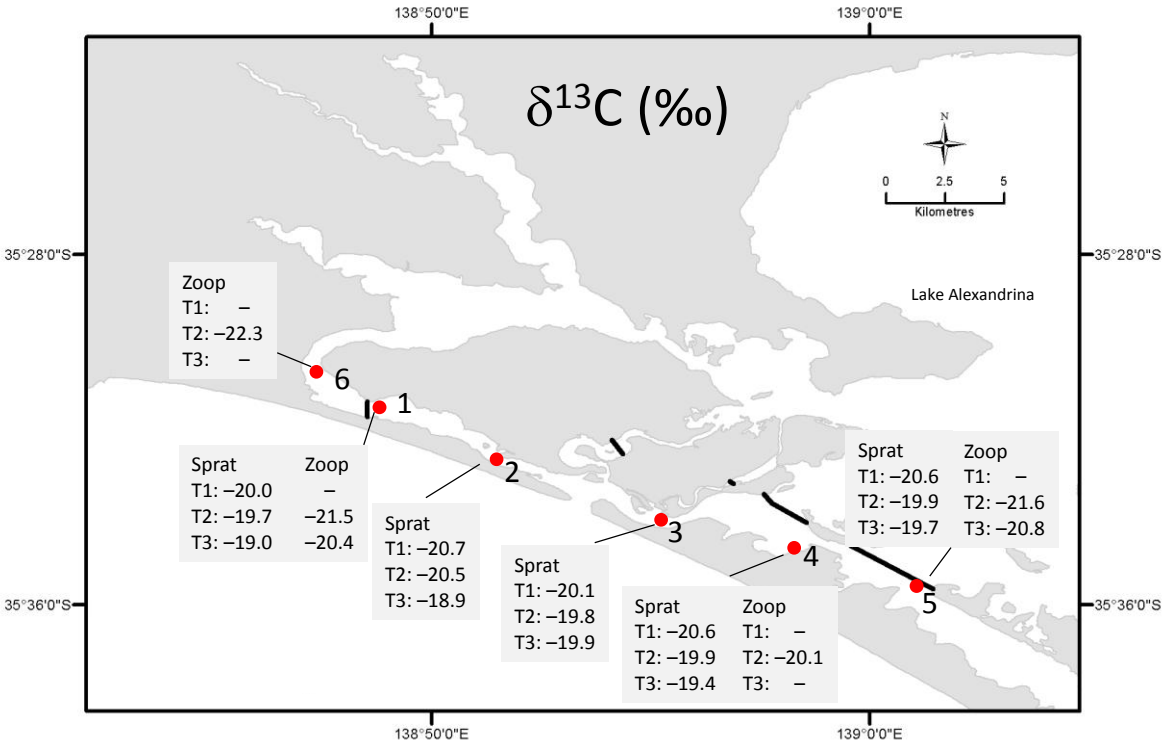

Figure 21. Mean values of $\delta^{13}C$ ratios (‰) from sandy sprat ('sprat') and zooplankton ('zoop') collected from sites 1–6 from Trip 1–3 (T1–T3).

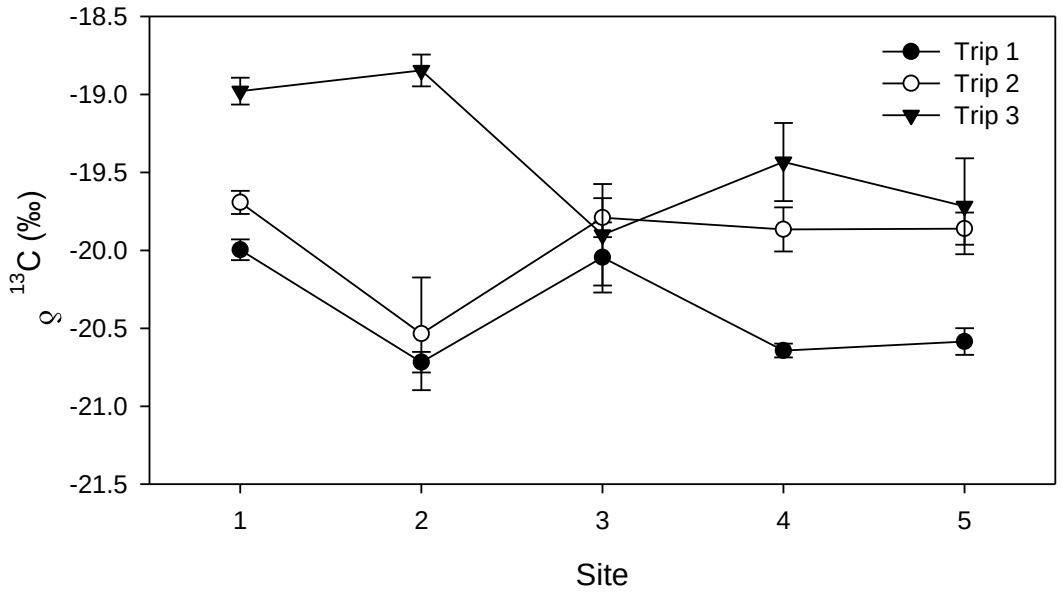

Figure 22. Mean values of $\delta^{13}C$ ratios (‰ ± SE) from sandy sprat collected at sites 1– 5 during trips 1–3. Sites presented from left to right (1–5) represent increasing distance from Goolwa Barrage.

Nitrogen

Mean $\delta^{15}N$ for sandy sprat collected from sites in the Murray estuary varied from 8.4‰ at Goolwa downstream during trip 3 to 11.8‰ at Tauwitchere during trip 1, higher than values for zooplankton from the Murray estuary (5.2 to 7.2‰) and the zooplankton sample from Goolwa upstream during trip 2 (4.4‰; Figure 23). PERMANOVA showed a significant difference in $\delta^{15}N$ between trips (*Pseudo-*$F_{2, 72}$ = 20.96, $p < 0.001$) and between sites (*Pseudo-*$F_{4, 72}$ = 3.20, $p = 0.027$), and a significant interaction between trip and site was evident (*Pseudo-*$F_{8, 72}$ = 9.73, $p < 0.001$). This suggests that values of $\delta^{15}N$ changed between sampling events, but that change was not consistent across sites. Sandy sprat collected from both Godfrey's Landing and Tauwitchere had significantly depleted $\delta^{15}N$ signatures during trip 3 ($p \leq 0.011$ for both comparisons; B-Y corrected $\alpha = 0.015$) and whilst non-significant, sandy sprat collected from Goolwa downstream and Ewe Island exhibited a similar pattern. Alternatively, sandy sprat collected from Rushy Island were significantly enriched in nitrogen during trip 3, relative to trips 1 and 2 ($p \leq 0.011$ for both comparisons).

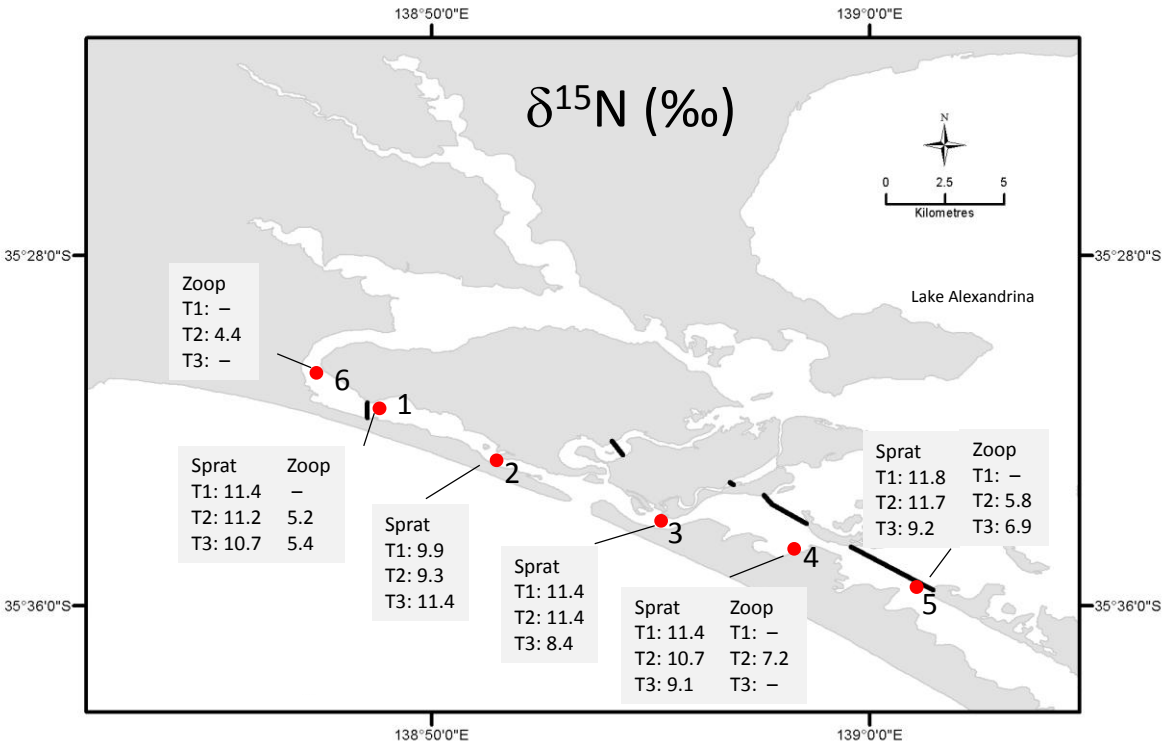

Figure 23. Mean values of $\delta^{15}N$ ratios (‰) from sandy sprat ('sprat') and zooplankton ('zoop') collected from sites 1–6 during sampling trips 1–3 (T1–T3).

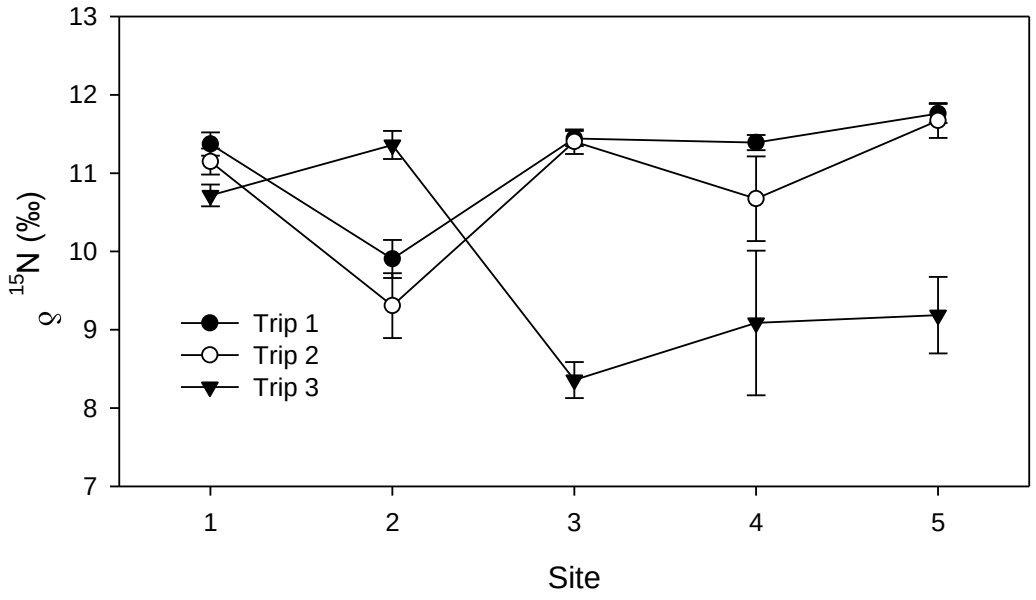

Figure 24. Mean values of $\delta^{15}N$ ratios (‰ ± SE) from sandy sprat collected at sites 1–5 during sampling trips 1–3. Sites presented from left to right (1–5) represent increasing distance from Goolwa Barrage.

Sulfur

Mean $\delta^{34}S$ for sandy sprat sampled from sites in the Murray estuary ranged from 13.1‰ at Godfrey's Landing during trip 3, to 17.3‰ at Goolwa downstream during trip 2. These values were higher than the mean $\delta^{34}S$ for zooplankton from Goolwa upstream during trip 2 (11.3‰) and zooplankton from Goolwa downstream during trip 3 (11.6‰; Figure 25). Note that sufficient zooplankton material for $\delta^{34}S$ analysis was only available at Goolwa upstream during trip 2 (1 replicate) and Goolwa downstream during trip 3 (3 replicates). As for $\delta^{13}C$ and $\delta^{15}N$, PERMANOVA showed a significant difference in $\delta^{34}S$ signature between trips (*Pseudo-*$F_{2, 72}$ = 13.32, $p < 0.001$) and between sites (*Pseudo-*$F_{4, 72}$ = 26.83, $p < 0.001$), and a significant interaction between trip and site was evident (*Pseudo-*$F_{8, 72}$ = 10.10, $p < 0.001$). This suggests that values of $\delta^{34}S$ changed between sampling events, but that change was consistent across sites. Pairwise testing of mean values of $\delta^{34}S$ showed that there was no significant difference between among trips at most sites, with the exception of Goolwa downstream, which was significantly enriched during trip 2 (p = 0.011; B-Y corrected α = 0.015), and Godfrey's Landing, which was significantly depleted during trip 3 (p = 0.013; Figure 26). Goolwa downstream had consistently high sprat $\delta^{34}S$ (16.3 to 17.3‰) whilst Ewe Island consistently had lower values (14.0 to 14.6‰).

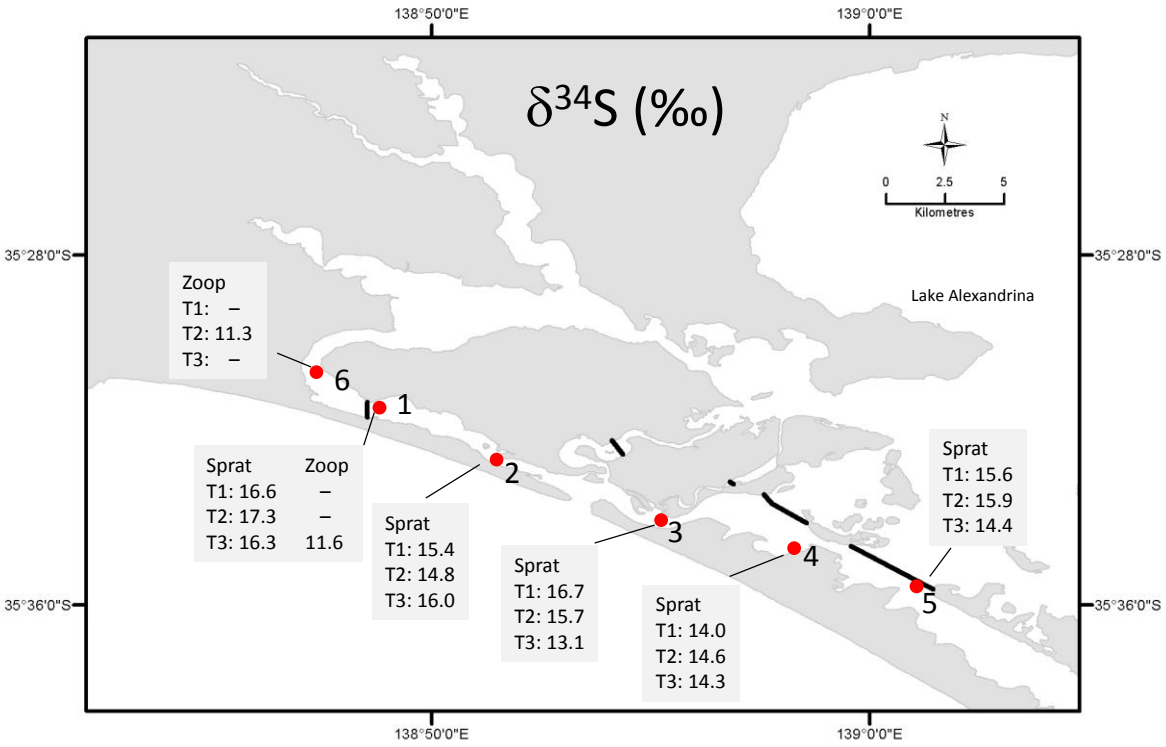

Figure 25. Mean values of $\delta^{34}S$ ratios (‰) from sandy sprat ('sprat') and zooplankton ('zoop') collected from sites 1–6 during sampling trips 1–3 (T1–T3).

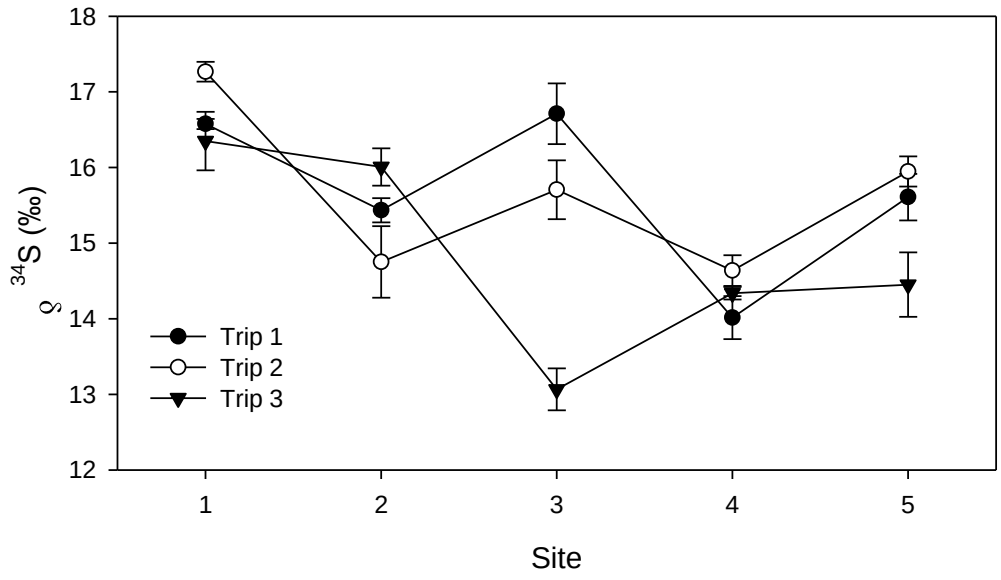

Figure 26. Mean values of $\delta^{34}S$ ratios (‰ ± SE) from sandy sprat collected at sites 1–5 during sampling trips 1–3. Sites presented from left to right (1–5) represent increasing distance from Goolwa Barrage.

Paired isotopes

The trends between trips were further explored by qualitatively comparing the average Murray estuary sandy sprat values (all sites combined) over time for pairs of isotopes. These values were also compared to sandy sprat isotope signatures collected in 2007 (Deegan *et al.* 2010) and 2013 (Johnson 2014). For C and N, the trend was for sandy sprat to become more enriched (less negative) in $\delta^{13}C$ and more depleted in $\delta^{15}N$ over time (from trip 1–3) in the current study (Figure 27a). When compared to $\delta^{13}C$ signatures for sandy sprat from 2007 (Deegan *et al.* 2010), a period of minimal freshwater discharge, signatures recorded in 2014 were substantially depleted (1.5–3‰). Likewise sandy sprat in 2007 had a more depleted $\delta^{34}S$ signature (S. Lamontagne, unpublished data; Figure 27b). Additionally, $\delta^{13}C$ signatures in 2014 were similar to those from 2013 (Johnson 2014), a year with generally similar hydrology to the current study.

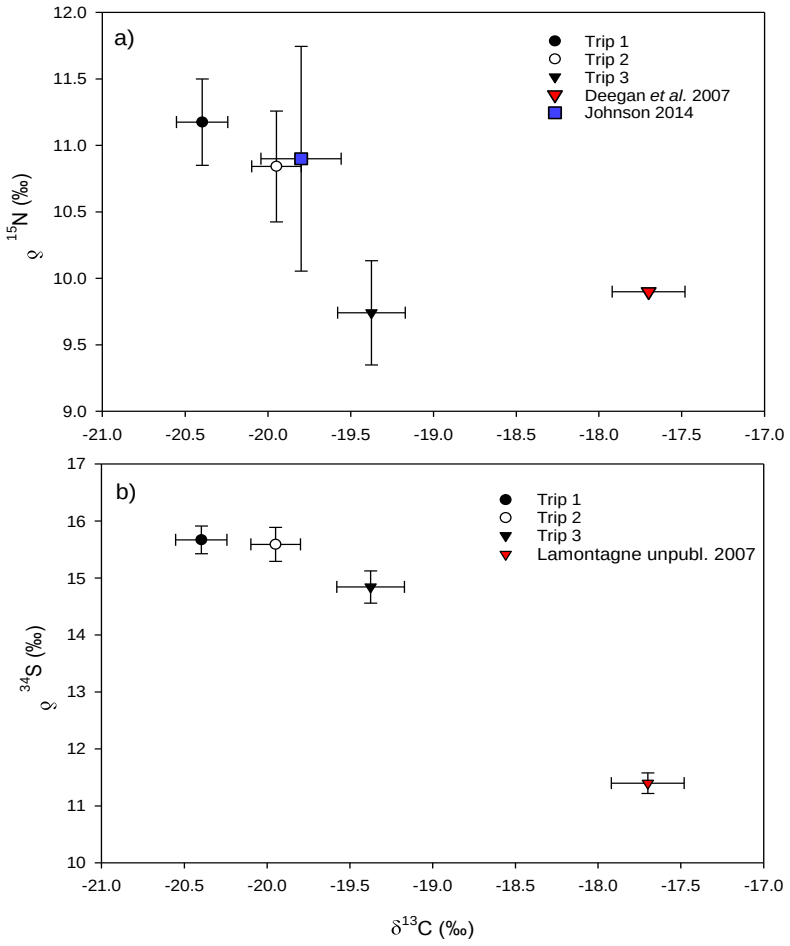

Figure 27. Mean values (± SE) of a) $\delta^{15}N$ vs $\delta^{13}C$ (‰) and b) $\delta^{34}S$ vs $\delta^{13}C$ from sandy sprat tissue samples across trips (all sites combined). Two added data points represent the $\delta^{15}N$ vs $\delta^{13}C$ isotopic signatures of sandy sprat collected from the Murray estuary in 2007 (Deegan *et al.* 2010) and 2013 (Johnson 2014).

Compound-specific stable isotope analysis

The trophic level of sandy sprat during trip 1, as calculated by comparing $\delta^{15}N$ signatures of glutamic acid and phenylalanine, ranged from 2.6 at Ewe Island to 3.1 at Goolwa downstream and Tauwitchere (Table 9). As a secondary consumer, the trophic level of sandy sprat would be assumed to be 3. There are no bulk $\delta^{15}N$ estimates for zooplankton for trip 1, but signatures varied from 4.4–7.2‰ during later trips and thus, the estimated $\delta^{15}N$ signature at hypothesised trophic level 2 (i.e. zooplankton as a primary consumer) was not constant in space or time. Using sites where both sprat and zooplankton bulk $\delta^{15}N$ could be measured during trips 2 and 3, sandy sprat trophic level was calculated (i.e. trophic level = [sprat $\delta^{15}N$ – zooplankton $\delta^{15}N$]/3.3 + 2) to range 2.7–3.8. Therefore, it appears CSIA provided a more accurate depiction of trophic position than traditional SIA.

Table 9. $\delta^{15}N$ values for the amino acids glutamic acid and phenylalanine, and mean trophic position of sandy sprat (± SE) collected from the Murray estuary during trip 1. Trophic level was calculated from these values as detailed in section 2.6.

	Mean ± SD		Mean trophic position ± SD
Site	**Glutamic acid**	**Phenylalanine**	
Goolwa Barrage	21.6 ± 0.9	2.1 ± 0.7	3.1 ± 0.1
Rushys Island	18.9 ± 0.5	1.0 ± 0.9	2.9 ± 0.1
Ewe Island	15.1 ± 0.1	-0.1 ± 0.2	2.6 ± 0.0
Tauwitcherie	22.7 ± 0.3	3.4 ± 0.7	3.1 ± 0.1

Given the novel nature of CSIA and limited data on source signatures, we compare the $\delta^{13}C$ signatures from sandy sprat for the essential amino acids isoleucine, leucine and lysine with published data for primary producers (e.g. marine algae, freshwater algae, bacteria, fungi, etc.) (Larsen *et al.* 2009 and 2013) (Figure 28). Sandy sprat $\delta^{13}C$ signatures usually did not directly match 'known' primary producers and there was substantial spatial variation in $\delta^{13}C$ signatures for sandy sprat. Nonetheless, $\delta^{13}C$ phenylalanine values (one of the 'essential' or isotopically conservative amino acids) in sandy sprat tissue were considerably depleted during trip 1 (–31 to –26‰; Table 10), relative to primary producer signatures from the literature (Figure 28)

Table 10. $\delta^{13}C$ values for the amino acids leucine, isoleucine, lysine, glutamic acid and phenylalanine from sandy sprat collected from the Murray estuary during trip 1.

Site	Leucine	Isoleucine	Lysine	Glutamic acid	Phenylalanine
Goolwa Barrage	-28.4	-37.4	-16.7	-23.6	-31.4
Rushys Island	-26.7	-29.2	-29.1	-11.4	-28.1
Ewe Island	-26.4	-43.9	-31.1	-12.3	-27.3
Tauwitcherie	-25.0	-36.4	-14.5	-11.1	-26.1

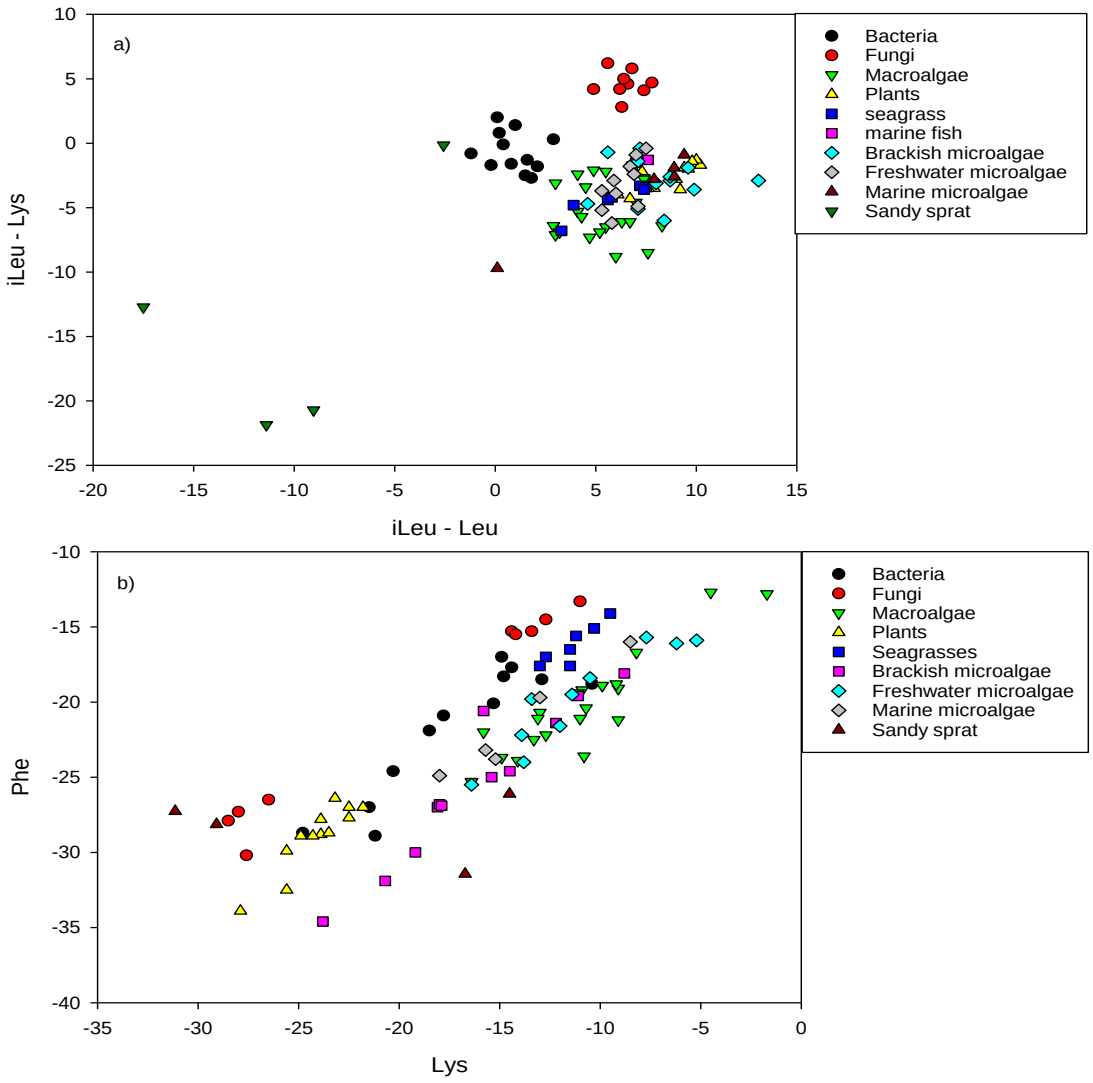

Figure 28. Compound specific amino acid carbon isotope data as a) a bi-plot of isoleucine-Leucine against isoleucine-Lysine and b) a bi-plot of Lysine against Phenylalanine. Data from sandy sprat collected from the Murray estuary during trip 1 is plotted with data from Larsen *et al.* (2013). Note: Brackish, Fresh and marine microalgae designations are based on culture conditions noted in Larsen *et al.* (2009).

4 Discussion

The use of environmental water allocations to achieve ecological outcomes in the estuaries of regulated rivers is becoming increasingly common (Adams 2014). Nonetheless, achieving positive ecological outcomes from such approaches is reliant on knowledge of the association of key ecosystem processes with freshwater discharge (Arthington *et al.* 2006). Understanding of the role of freshwater discharge in regulating water level, hydrological connectivity and salinity regimes in the Coorong, and subsequent influences on estuarine biota, has improved in recent years (Brookes *et al.* 2009, Zampatti *et al.* 2010, Dittmann *et al.* 2013b). Nevertheless, knowledge of the influence of freshwater discharge on estuarine productivity, through organic matter transport and trophic subsidy, remains limited.

The current study investigated the influence of low-volume freshwater discharge (<25,000 $ML.d^{-1}$) in 2014 on water physico-chemistry, primary productivity (i.e. phytoplankton community composition and abundance), microbiota community structure (e.g. zooplankton), and the diet and freshwater derived trophic subsidy of sandy sprat. All parameters investigated exhibited intra-annual patterns associated with declining freshwater discharge through 2014, and inter-annual variability when compared with previous data from years of disparate hydrology (e.g. drought and flood). In particular, the current study has, for the first time, demonstrated that organic matter exported to the Coorong with freshwater discharge from the Murray Barrages contributes materially to estuarine productivity. The findings are discussed in regards to individual project components and are synthesised in the context of implications for ecosystem management.

4.1 Water quality and phytoplankton community structure

Patterns of increasing salinity across sampling trips, and with distance from discharge points, indicated the influence of decreasing discharge on water physico-chemistry in the Murray estuary. These patterns suggest that following the elevated discharge event of August, seawater exchange through the Murray Mouth likely exceeded the subsequent low–volume discharge through the barrages. Nonetheless, changes in salinity were not consistent across sites; salinity increased at a slower rate at Goolwa downstream and Rushy Island, compared to Tauwitchere, and was attributed to the greater influence of low-volume discharge at these sites due to the narrowness of the receiving channel downstream of Goolwa Barrage. Patterns of spatio-temporal variability in salinity were reflected in nutrient and phytoplankton dynamics.

The nutrient and phytoplankton data were interpreted as indicating that sites in the vicinity of Goolwa and Tauwitchere barrages were significantly active transformation zones and that freshwater organisms delivered with discharge were either recycled through biogeochemical cycles that released dissolved nutrients into the water column or used as food resources. At Tauwitchere, dissolved nutrients were transported to Ewe Island, as indicated by elevated concentrations at this site. In contrast, dissolved nutrients were not transported from Goolwa downstream to Rushy Island, but instead total nutrient concentrations increased at this site indicating incorporation of dissolved nutrients into growing organisms and their transport. This suggestion was supported by the distribution of chlorophyll-a, which was particularly high at the Goolwa downstream site (4–10 times greater than other sites). Total phytoplankton numbers were also greatest at Goolwa downstream with significant contributions from dinoflagellates and cryptophytes that were considered of marine/brackish origin as they had not appeared in lake samples. This suggests Goolwa downstream was a zone of high productivity. At all sites, cyanobacteria dominated the phytoplankton communities, likely transported from upstream with freshwater discharge and although declining in number, did not disappear as rapidly as the microalgae from the lake. Nonetheless, continued declines in numbers were observed in response to increasing salinity. This reflects the ability of some cyanobacteria to tolerate saline conditions. In this case growth was not evident and the prolonged occurrence of the cells and slower rate of decline was attributed to a more robust cell wall. *Aphanocapsa* was the dominant cyanobacteria and its growth is severely curtailed as salinities increase above ~30,000 $\mu S.cm^{-1}$ (20 $g.L^{-1}$) (Ifeanyi *et al.* 2011). Conductivity was demonstrated to be a major driver of the changes in phytoplankton community composition across the sites and across trips. Increasing salinity was also aligned with reductions in nutrients.

4.2 Zooplankton community structure

Identification and enumeration of zooplankton samples yielded 31 taxa comprising rotifers, cladocerans, copepods, ostracods, amphipods and decapods. Molecular analyses identified the presence of many of the same taxa, and many more planktonic taxa (e.g. dinoflagellates, turbellarians, etc.), which was expected given this approach is likely to detect a broader range of biota, including small microeukaryotes not sampled with traditional methods. Nonetheless, both analyses identified a suite of both freshwater and estuarine/marine organisms, and similar patterns of spatio-temporal variability in community structure in association with discharge and salinity.

The zooplankton diversity and abundances recorded during this study were comparable to those found during a period of low discharge (peak ~14,000 ML.d^{-1}) in 2003 (Geddes 2005) and considerably lower than those found during high discharge (>80,000 ML.d^{-1}) in 2010/11 (Shiel and Aldridge 2011, Shiel and Tan 2013). Unfortunately no comparable data was available for the extended no-flow period from 2007–2010. Despite similarities in density, community composition in 2014 differed from that of 2003. During low flows in 2003, zooplankton communities at comparable sites to those investigated in this study were primarily comprised of freshwater copepods (Geddes 2005). In contrast, during comparable discharge in the current study, these species were rare whilst freshwater (e.g. *Keratella australis* and *Filinia* sp) and estuarine rotifers (e.g. *Synchaeta triophthalma*) were abundant.

Antecedent hydrology typically has a large influence on zooplankton community composition and differences in community structure highlighted between the current study and that of Geddes (2005a) likely reflects contrasting antecedent hydrology and different water residence times (WRT) within Lake Alexandrina prior to barrage releases. Prior to the barrage releases of 2003, the barrages had remained closed for a period of 630 days, resulting in a long WRT. In contrast, whilst discharge to the Coorong in 2014 was low in a historical context, discharge had been continuous since September 2010, likely resulting in shorter WRT. Long WRT has a strong positive relationship with zooplankton abundance and biomass, and results in a shift from rotifer to crustacean (primarily copepods and cladocerans) dominated communities (e.g. Basu and Pick 1996, Baranyi *et al.* 2002, Obertegger *et al.* 2007), and was the likely mechanism for disparity between these two studies.

Zooplankton community structure differed across trips at most sites and was primarily characterised by decreasing abundance of the freshwater cladoceran *Bosmina meridionalis* and freshwater rotifers (*P. tentaculatus, F. longiseta* and *F. pejleri*), and increased abundance of copepod nauplii over time. This pattern was most prevalent downstream of Goolwa Barrage, although species of freshwater origin were also sampled in considerable abundance downstream of Tauwitchere Barrage. Godfrey's Landing, the site furthest from freshwater influence, typically exhibited the most estuarine/marine community. As such, a general pattern of low-volume freshwater discharge influencing zooplankton community composition within the vicinity of discharge was evident, but with a declining magnitude of influence across trips. This pattern of spatio-temporal variability in zooplankton community structure was also reflected in the diet of sandy sprat.

4.3 Variability in sandy sprat abundance

Sandy sprat was abundant in the Murray estuary in 2014 relative to the period of no freshwater discharge from 2007–2010. Indeed, the species dominated the assemblage, comprising ~60% of the total catch recorded by Bice and Zampatti (2015) adjacent Goolwa and Tauwitchere barrages. Nevertheless, abundance was less than that observed following high freshwater discharge in 2011/12. As such, patterns of abundance continued to indicate an association with freshwater discharge. Intra-annual variability in abundance also corresponded with that observed in previous years, with greatest abundance in November and December, and lower abundance thereafter (SARDI unpublished data).

4.4 Diet of sandy sprat

The diet of sandy sprat, as indicated by both identification and enumeration, and molecular analyses of gut content, included a diverse range of zooplankton taxa and indicated the importance of crustaceans. Molecular analyses also highlighted smaller contributions to sandy sprat diet by other groups of organisms not detected during identification and enumeration, including dinoflagellates, bivalves and turbellarian worms. Both sets of analyses indicated that diet varied considerably between sites and trips, consistent with decreasing discharge and rising salinity. The prevalence of freshwater organisms (e.g. *Bosmina meriodinalis* and unidentified Daphiids) in gut content of sandy sprat was greatest within proximity of discharge at Goolwa downstream and Tauwitchere, but typically decreased across trips, with increasing importance of estuarine/marine organisms (e.g. copepods).

The greatest numbers of prey items were recorded from the gut of fish from Goolwa downstream and Ewe Island, sites where zooplankton abundance was typically high. Sandy sprat, however, did not feed indiscriminately, but selected for specific organisms. This included a strong preference for the freshwater cladoceran species *Bosmina meriodinalis*, a species commonly found in riverine and lake environments including the River Murray and Lake Alexandrina (Geddes 1984a). This was particularly evident during trip 1, the first sampling event following elevated discharge in August 2014. Amphipods and harpacticoid copepods, both groups of benthic estuarine invertebrates, were also selectively preyed upon, with harpacticoid copepods being the most dominant prey item across the study. Nevertheless, given zooplankton sampling is likely biased towards pelagic rather than benthic organisms, the Strauss index for this group may have been artificially elevated. These results, however, are consistent with unpublished data indicating that harpacticoid copepods (present in 73 % of

individuals) and amphipods (present in 59% of individuals) were also the most common prey items of sandy sprat from the Coorong in 2013 (Hossain Pers Comm).

The dominance of these benthic invertebrates in the diet of sandy sprat highlights a link between the benthic and pelagic food web within the Murray estuary. The Coorong is relatively shallow and exposed to both wind and tidal water movement; factors that generate pulsing currents that can strip microorganisms from the benthos (Munro *et al.* 1978) potentially increasing their susceptibility to predation. Additionally, it is possible that freshwater flows not only provide a direct subsidy of food resources for sandy sprat in the form of species such as *B. meridionalis* and *Keratella australis,* but they may also provide the organic subsidies required to sustain components of the benthic invertebrate community.

Prolonged low flow may result in the depletion of benthic organic resources and in turn benthic organisms. Indeed, long-term monitoring within the Coorong and Lower Lakes region demonstrated declines of macroinvertebrate species, including amphipods, during drought (Geddes 1987, Geddes 2005, Dittmann *et al.* 2006), and alternatively, increased abundance and distribution following recommencement of discharge in 2010/11 (Dittmann *et al.* 2012, Dittmann *et al.* 2013a, Dittmann *et al.* 2013c). Maintaining a balance between high freshwater flows and tidal influences may be important for maintaining benthic and pelagic coupling and thus, food web structure within the Coorong and Murray estuary.

4.5 Trophic subsidy

Analysis of isotopic signatures provided insight on the interaction of sandy sprat and zooplankton, and the influence of hydrology on trophic dynamics, particularly when considered in the context of previous studies in the Coorong and the broader literature. In concurrence with results from gut content analyses, enrichment of $\delta^{15}N$ signatures between zooplankton and sandy sprat highlighted their trophic positions as primary and secondary consumers, respectively. In addition, temporal variability (both intra- and inter-annual) in $\delta^{13}C$ and $\delta^{34}S$ signatures provided insight on the origin of organic matter contributing to the diet of sandy sprat.

Estuarine food-webs are supported by organic matter derived from a combination of freshwater, estuarine and marine sources (Peterson *et al.* 1986). These three sources can be discriminated using their $\delta^{13}C$ and $\delta^{34}S$ signatures because marine sources tend to have an enriched $\delta^{34}S$ signature (~20‰, similar to marine sulfate), freshwater sources tend to have a depleted $\delta^{13}C$ signature and estuarine benthic sources tend to have a highly depleted $\delta^{34}S$ signature. This pattern is evident in the Coorong (Figure 29). Lamontagne *et al.* (2007) found

that macro- and filamentous algae at Pelican Point, during a period of limited freshwater inflow in 2005, had a marine-like signature ($\delta^{13}C$ ~ –18‰; $\delta^{34}S$ ~20‰). Fish in the Coorong at that time had a wide range of signatures, but $\delta^{34}S$ signatures were generally lower, suggesting the utilisation of an estuarine-derived benthic food source. The signature of this 'benthic' food source can be approximated by *Capitella*, the most abundant marine worm in the Coorong (Rolston and Dittmann 2009). In 2007, during a period with no freshwater inflow, *Capitella* had a $\delta^{13}C$ of ~ –13‰ and $\delta^{34}S$ of ~8‰ (Deegan *et al.* 2010, Lamontagne Unpublished data). In contrast, the signature of the zooplankton samples collected from upstream of Goolwa Barrage in the current study was consistent with trends expected for organic matter from a 'freshwater' environment, especially a more depleted $\delta^{13}C$ (–23‰) relative to the other sources.

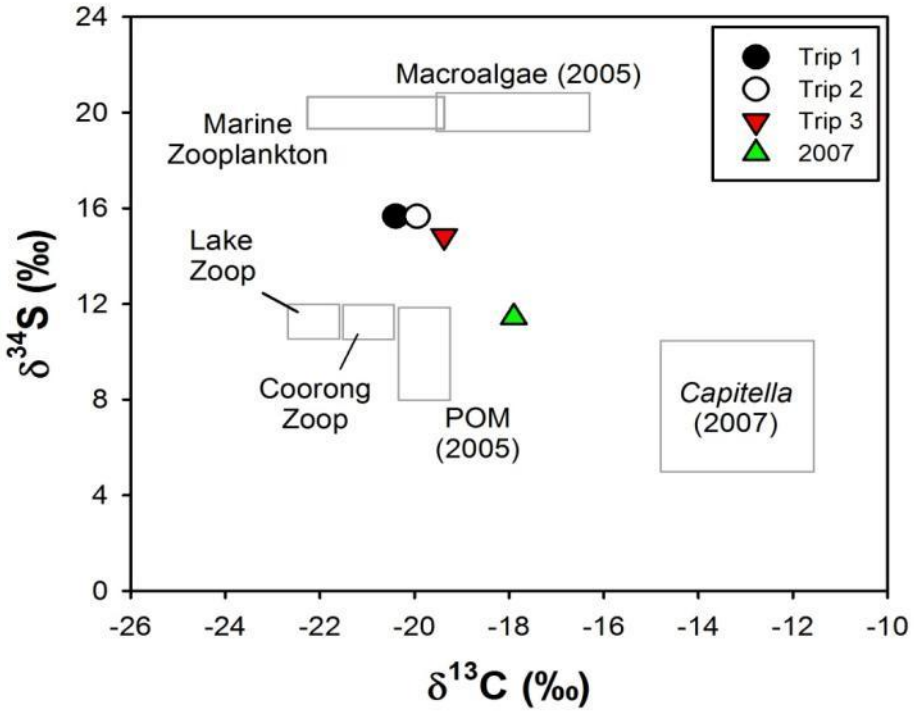

Figure 29. Variations in $\delta^{13}C$ and $\delta^{34}S$ in sandy sprat and potential food sources in the Coorong region. *Capitella* signature (a common marine worm in the Coorong) from Deegan et al. (2010) and Lamontagne (Unpublished data) and macroalgae and Particulate Organic Matter (POM) signatures from the Coorong from Lamontagne et al. (2007).

As for most fish in the Coorong (Lamontagne *et al.* 2007, Deegan *et al.* 2010), there is little to no direct overlap in isotopic signature between sandy sprat and the three hypothesised organic matter sources available in the system. However, sandy sprat isotopic signatures are intermediate between these organic matter sources, suggesting that a mixture is used (Figure 29). Moreover, the shifts in sandy sprat isotopic signature over time are consistent with the

likely availability of different sources of organic matter over time. For example, during the Millennium drought and prolonged low discharge in 2007, sandy sprat isotopic signatures, particularly $\delta^{34}S$, were closer to *Capitella* (Deegan *et al.* 2010, Lamontagne Unpublished data). This suggests a greater reliance on organic matter produced within the Coorong in the absence of freshwater discharge. In contrast, during trip 1 in this study, approximately two months following an unregulated flow event of >20,000 $ML.day^{-1}$ in August, sandy sprat signatures were typically more similar to zooplankton from upstream of Goolwa Barrage, with mean $\delta^{13}C$ across sites depleted by ~3‰ relative to 2007. Post trip 1, sandy sprat signatures at most sites gradually became more enriched and 'drifted' towards *Capitella* from 2007, with increasing time from the August flow event, again suggesting a greater reliance on organic matter derived from within the Coorong. This does not mean that zooplankton from the Lower Lakes were not consumed during trips 2 and 3, but that with decreasing discharge, they provided an overall smaller contribution to the diet of sandy sprat. This pattern is generally consistent with the gut content analyses, which indicated a general decline in the prevalence of freshwater derived organisms, particularly the cladoceran *Bosmina meridionalis* following Trip 1 and relatively greater contribution of Coorong-derived organisms (e.g. harpacticoid copepods) during subsequent trips. Overall, isotopic signatures in sandy sprat are consistent with their diet being subsidised by carbon of freshwater origin. This may take the form of organic matter transported from upstream being incorporated into the estuarine food web (e.g. grazing of organic matter by harpacticoid copepods and then predation by sandy sprat) or via direct diet subsidy of sandy sprat through predation upon organisms exported from Lake Alexandrina to the Coorong.

CSIA, using amino acids, produced mixed results. Calculation of trophic position of sandy sprat using this technique produced a narrower range of values than traditional bulk SIA, and thus, it appears to offer some advantages for this purpose. Alternatively, the $\delta^{13}C$ signatures of amino acids from sandy sprat were highly variable when compared with values for primary producers in the literature (Larsen *et al.* 2009 and 2013). Substantially depleted $\delta^{13}C$ signatures of sandy sprat phenylalanine, however, provide an indication of a substantial freshwater source for this essential amino acid (Larsen *et al.* 2009). Nonetheless, limited understanding of the compound-specific isotopic signatures of organic matter sources in the Coorong dictates this technique requires further trialling to determine its applicability.

In summary, bulk SIA provided an indication of the source of organic matter consumed by sandy sprat and has helped elucidate why fish isotopic signatures vary in the Coorong. Sandy sprat are typically considered a marine migrant, which spawns in the marine environment, and specifically in South Australia, in both Gulf St Vincent and Spencer Gulf (Rogers and Ward

2007), before entering the Coorong as larvae/juveniles. Based on our results we have developed a hypothesised conceptual model of variability in stable isotope signatures based on migration and hydrology. We surmise that during migration in and out of the Coorong, sandy sprat isotopic signatures vary in a counter clockwise fashion (Figure 30). When migrating into the Coorong, individuals most likely have a 'marine-like' isotopic signature (i.e. enriched $\delta^{34}S$). When River Murray inflows occur, sandy sprat isotopic signatures will tend to drift towards freshwater organic matter (i.e. depleted $\delta^{13}C$). When inflows stop or are reduced, isotopic signatures will tend to drift towards the 'Coorong' benthic organic matter end-member (depleted $\delta^{34}S$ and enriched $\delta^{13}C$). Eventually, as individuals migrate back to the Southern Ocean, they will gradually reacquire a more 'marine-like' isotopic signature. How far sprat will 'drift' towards a given end-member will depend on the relative proportion of prey consumed, the length of their stay in the Coorong and tissue turnover rates. Three questions need to be answered to refine this conceptual model:

1) How do the isotopic signatures of freshwater organic matter vary as a function of River Murray discharge?
2) What is the tissue turnover rate for sandy sprat over time (i.e. how long does it take for sandy sprat to acquire a new isotopic signature)?
3) What is the 'marine' isotopic end-member for sandy sprat (i.e. what is their isotopic signature when they enter the Coorong)?

The greatest current uncertainty relates to how the signature for freshwater-derived $\delta^{13}C$ varies with freshwater discharge. Carbon isotopic signatures of organic matter are known to vary with riverine hydrology due to variable connectivity and differing inputs of autochthonous and allochthonous materials (Aspetsberger *et al.* 2002). Therefore, there should broadly be two different freshwater-derived organic matter end-members entering the Coorong; one source will be typical for low flows and the other for higher flows (Aldridge and Brookes 2011), also corresponding to the 'clear' and 'turbid' ecological states found in the Lower Lakes (Geddes 1984a, b). Under low flow conditions, when River Murray water transits in the Lower Lakes for months to years, the organic matter exported will likely be primarily derived from Lake Alexandrina itself (in the form of plankton). Early during floods, river inflows to Lake Alexandrina will also tend to 'push' old lake water into the Coorong. However, in the mid-later stages of floods, organic matter from the river channel and floodplains will be exported, potentially promoting isotopic signatures different to lake-derived organic matter (Peterson *et al.* 1986). Variability in the isotopic signatures of freshwater end-members, in association with variable hydrology, requires further investigation.

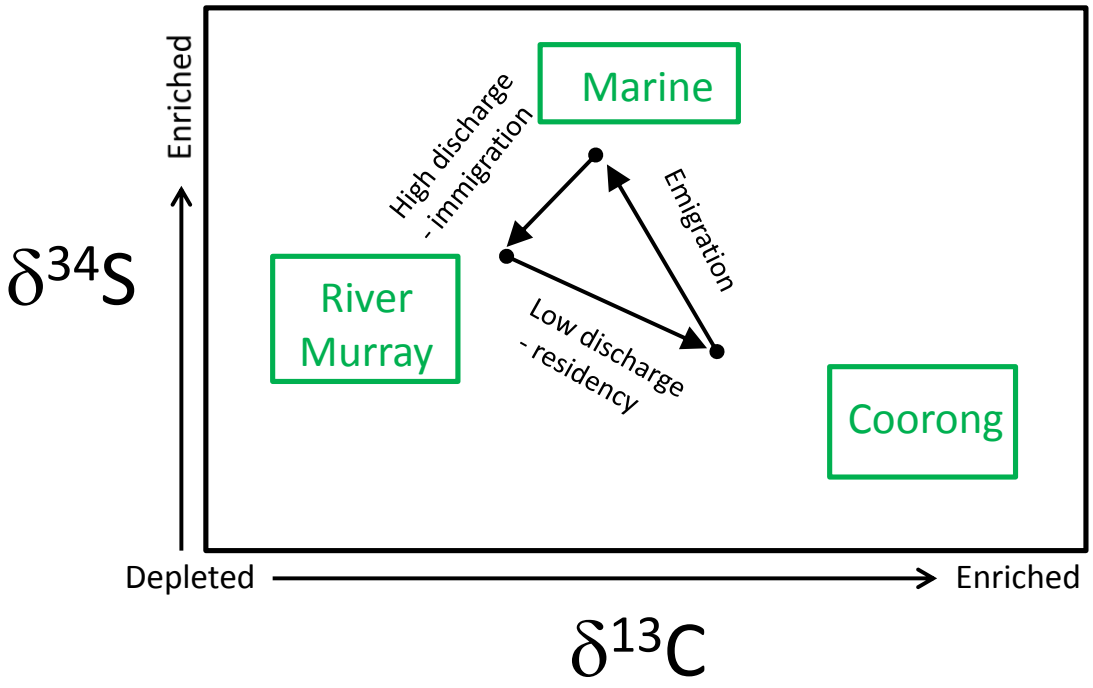

Figure 30. Conceptual model of variability of sandy sprat isotopic signatures (i.e. $\delta^{34}S$ *vs* $\delta^{13}C$) as a function of its migration in and out of the Coorong and the availability of freshwater-derived organic matter in the Coorong.

4.6 Synthesis and conclusions

The current study provides evidence of trophic subsidy as a potential causal link between high abundance of sandy sprat and freshwater discharge. Indeed, a significant portion of the diet of sandy sprat was derived from organic matter and biota (e.g. *Bosmina meridionalis*) exported to the Murray estuary from the freshwater Lower Lakes. As such, we have demonstrated for the first time that the organic matter exported with freshwater discharge contributes materially to productivity in the Murray estuary. Additionally, prey consumption by sandy sprat generally reflected varying abundance of a suite of preferred prey species and thus, the species appears a suitable general indicator for the origin of organic matter fuelling the Coorong food-web.

Similar associations between high abundance of larval/juvenile life stages of estuarine associated fishes and freshwater discharge have been observed in other semi-arid estuaries (Martin *et al.* 1992, Whitfield 1994). Martin *et al.* (1992) recorded a 15-fold increase in the abundance of the estuarine clupeid *Gilchristella aestuaria*, a closely related and similar species

to sandy sprat, in the St Lucia estuary in South Africa, immediately following freshwater flushing of the estuary and in association with phytoplankton and zooplankton blooms. The current study, however, provides empirical evidence of this trophic linkage confirming both predation of freshwater zooplankton by sandy sprat and evidence of trophic subsidy from a freshwater source. This work adds to a growing body of literature on the importance of terrestrial and/or freshwater derived carbon in estuarine and marine fish production (Darnaude *et al.* 2004, Darnaude 2005, Kostecki *et al.* 2010)

Determining the degree to which freshwater-derived organic matter drives fish production in the Coorong is a more complex problem than demonstrating that organic matter is used to some extent. It involves understanding growth and mortality rates, food consumption and assimilation rates, for sandy sprat and other organisms. It is possible to define boundaries for fish productivity in an ecosystem using bioenergetics (Brandt and Hartman 1993), but this approach has received little attention to date in Australia. Nonetheless, recently improved understanding of trophic relationships in the Murray estuary provides some indication of likely general fish production benefits from enhanced sandy sprat abundance.

Gut content analyses of juvenile mulloway and Australian salmon confirmed that sandy sprat was among the most important prey items for these fishes in the Murray estuary in 2013/14 (Giatas and Ye 2015). Indeed, sandy sprat were found in the gut content of ~63% of juvenile mulloway sampled, with the number of individuals per gut ranging from 0–49 individuals, and an average of 9 sandy sprat per mulloway. Thus, growth of mulloway in the Coorong and potentially survival, and recruitment to larger size classes, appear in part supported by sandy sprat. Enhanced recruitment of mulloway and subsequent year class strength are associated with freshwater discharge to the Coorong (Ferguson *et al.* 2008). Whilst, this may relate to multiple factors, including stimulation of spawning aggregations and provision of favoured salinity regimes, the provision of abundant preferred prey resources, including sandy sprat, during years of freshwater discharge, likely contributes. In support of this hypothesis, during the recent period of no discharge from 2007–2010, sandy sprat were typically >100-fold less abundant than during years of freshwater discharge and concurrently, mulloway recruitment and abundance in the Coorong was limited (Earl and Ward 2014, Ye *et al.* 2014).

SIA undertaken on mulloway collected from the Murray estuary in March 2014 (Johnson 2014) demonstrated the trophic position ($\delta^{15}N$) and source of organic matter in mulloway tissue ($\delta^{13}C$). These analyses confirmed that juvenile mulloway, and indeed larger individuals (>400 mm), were top order predators, that likely preyed upon sandy sprat (Giatas and Ye 2015). This concurs with previous SIA of these two species from 2007 (Deegan *et al.* 2010). Perhaps of

most interest, however, was apparent depletion of $\delta^{13}C$ signatures in mulloway in 2013/14, relative to 2007, likely as a result of increased predation upon sandy sprat and the freshwater clupeid bony herring (*Nematalosa erebi*). This provides further evidence of the likely incorporation of freshwater derived carbon into the estuarine food web that was measureable at the top level of the food web.

Variability in the abundance of sandy sprat and mulloway, together with stable isotope, gut content and zooplankton community data, suggest changes in food web structure and ecosystem productivity with differing discharge. The data from the current study indicate sandy sprat may prey upon a variety of organisms depending upon availability. Other studies, present a similar pattern for mulloway (Geddes and Francis 2008, Deegan *et al.* 2010, Giatas and Ye 2015). Whilst sandy sprat diet was not investigated during high volume discharge during the years 2010–2013, sandy sprat were highly abundant, and zooplankton, including freshwater species determined to be important prey items in the current study, were substantially more abundant than in 2014 (Shiel and Aldridge 2011). Additionally, juvenile mulloway prey upon a variety of organisms including sandy sprat, and truly freshwater fishes such as bony herring, during high freshwater discharge, but diet is largely comprised of estuarine and marine organisms during low freshwater discharge. In general, these data suggest periods of low freshwater discharge are characterised by a food web driven by primary production occurring in estuarine and marine environments, with the diet of higher trophic levels largely supported by marine organisms, and resulting in overall low secondary productivity. Alternatively, periods of high freshwater discharge, are characterised by a food web driven by primary production occurring in freshwater and estuarine environments, with the diet of higher trophic levels largely supported by freshwater and estuarine organisms, and resulting in high overall secondary productivity

Evidence of enhancement of secondary productivity, in particular commercially important species, highlights the socio-economic benefit of freshwater delivery to the Coorong. In 2012/13, the Lakes and Coorong commercial fishery was estimated to contribute $18.8 million to the Gross State Product of South Australia (Econsearch 2013). The potential enhancement of mulloway condition, recruitment and abundance through a direct trophic link with sandy sprat and increases in habitat availability for a range of other commercially harvested species (e.g. yellow-eyed mullet, *Aldrichetta forsteri*) during times of freshwater discharge, likely enhances the economic status of the Lakes and Coorong fishery.

4.7 Management implications

The current project presents empirical evidence of the direct influence of freshwater discharge on productivity and trophic subsidy in the Coorong. As such, it provides support for the delivery of similar volumes of environmental water in the future, including under the Murray-Darling Basin Plan. Discharge through the Murray Barrages over the 2014/15 'water year' (~860 GL) was relatively low, from both pre- and post-regulation perspectives (since 1940's, mean annual discharge is ~4700 GL). Notwithstanding, these volumes of water convey considerable amounts of freshwater derived organic matter and biota that elicit measurable trophic subsidy and enhanced productivity, relative to no flow periods.

There is considerable interest in how environmental water is delivered to the Coorong, in regards to variability in timing, location (i.e. different barrages) and volumes. Sampling in the current study took place during relatively stable, but gradually declining, low discharges (8–2400 ML.day^{-1}) in November–December, following a higher discharge event in August 2014 (peak ~23,000 ML.day^{-1}). Over the 23-day period from 3–25 August, ~337 GL was discharged to the Coorong, compared to ~233 GL over the subsequent 127-day period from 26 August–31 December 2014. Abiotic and biotic patterns indicated declining influence of freshwater discharge occurred in association with increasing time from the August high flow. This included increasing salinity, declining phytoplankton biomass, shifts in zooplankton community structure and shifts in stable isotope signatures of sandy sprat. The fact that these parameters exhibited these trajectories 60–90 days post the discharge event, suggests associated productivity benefits may be realised for months following such events. Thus, in a productivity and trophic subsidy context, the delivery of short-lived, relatively large discharge events may result in prolonged productivity responses. Continued sampling through subsequent months (January–March), after greater time had elapsed since the August flow, may have provided insight on the longevity of abiotic/biotic responses and allowed greater comparison between the benefits of conspicuous discharge events and consistent low discharge.

The current project was not designed to determine optimum timing and locations for water delivery, but provides some insight on these components of environmental water delivery. Sandy sprat abundance was greatest in October and November in the current study, a result consistent with previous monitoring in the Murray estuary (SARDI unpublished data), potentially reflecting recent migration from the Southern Ocean. As such, delivery of freshwater during spring may couple high productivity with incoming migration. Furthermore, peak flows to the Coorong during spring were a feature of the natural hydrograph of the lower River Murray (Maheshwari *et al.* 1995). The findings of the current study also suggest that

water delivered from either Goolwa or Tauwitchere Barrages deliver freshwater zooplankton to the Coorong that may be consumed by sandy sprat.

The timing, location and volume of discharge to the Coorong is currently managed to achieve various ecological objectives (e.g. salinity targets, maintaining connectivity, etc.) and at times, these objectives may be competing. Ultimately, all targets and objectives should be considered when determining favourable discharge hydrographs, but we suggest that where possible, the inclusion of conspicuous flow peaks, particularly during spring, may elicit the greatest productivity responses.

4.8 Recommendations for future research

This study provided evidence of the importance of freshwater discharge to trophic dynamics in the Murray estuary and the potential of sandy sprat as an ecological indicator for the sources of organic matter driving the food web of the Murray estuary, using a range of novel techniques. Thus, it may inform future research and here we provide suggestions in regards to refining understanding of trophic dynamics in the Coorong, and thus supporting environmental water allocations, and the refinement of research methods.

Determining the degree with which freshwater-derived trophic subsidy drives total fish production within the Coorong would greatly inform the delivery of environmental water allocations. This is a complex task, which may involve analyses of bioenergetics of sandy sprat and numerous other fishes. Investigations may include, but are not limited to,

- Determining the marine isotopic signature of sprat before entering the Coorong;
- Determining the composition and isotopic signature of freshwater-derived organic matter entering the Coorong under varying hydrological conditions;
- Evaluating differences in the quality of different food sources (e.g. freshwater vs estuarine/marine, rotifer vs copepod, etc.) and how these effect growth and survival of sandy sprat; and
- Evaluating changes in sprat population productivity, and potentially other species, over time (based on diet, biomass, changes in isotopic signature, etc.) using bioenergetics analyses.

Future research developing the use of both CSIA and molecular finger-printing would greatly benefit the above investigations and others. CSIA appears promising as a technique to study trophic dynamics in many ecosystems, including the Coorong, but its applicability is reliant on greater understanding of the isotopic signatures of key organic matter sources in the Coorong. The current downfall of molecular fingerprinting approaches is the limited ability to classify

sequences sampled in the field into taxonomic groups due to the imperfect nature of current databases. Nonetheless, increasing use of this technique will improve databases and capacity to classify organisms.

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6 Appendix

Appendix 1. Summary of proportional contributions of microbiota taxa to community composition from all sites and trips.

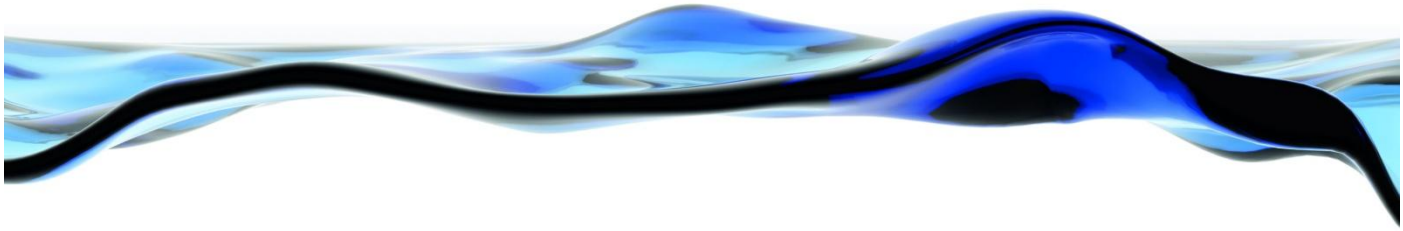
	Trip 1					Trip 2						Trip 3				
	Rushy Island	**Ewe Island**	**Godfrey's Landing**	**Tauwitchere**	**Goolwa downstream**	**Rushy Island**	**Ewe Island**	**Godfrey's Landing**	**Tauwitchere**	**Goolwa downstream**	**Goolwa upstream**	**Rushy Island**	**Ewe Island**	**Godfrey's Landing**	**Tauwitchere**	**Goolwa downstream**
Rotifera																
Brachionidae																
Brachionus calyciflorus	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
Brachionus calyciflorus complex sp.1 (small)	0	0	0	0	0	0	0	0	0	0	3	0	0	0	0	0
Brachionus calyciflorus complex sp.1 (large)	0	0	0	0	0	0	0	0	0	5	7	0	0	0	0	0
Brachionus keikoa	0	0	0	0	0	0	0	0	0	8	5	0	0	0	0	0
Keratella australis	0	0	0	12	20	5	0	0	11	30	23	0	0	0	0	10
Conochilidae																
Conochilus sp. *cf*	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
Filinidae																
Filinia pejleri	0	0	0	0	0	0	0	0	0	10	25	0	0	0	0	0
Filinia grandis	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0
Filinia longiseta	0	0	0	0	0	0	0	0	5	13	14	0	0	0	0	0
Filinia australiensis	0	0	0	16	4	0	0	0	0	0	0	0	0	0	0	0
Hexarthridae																
Hexarthra sp.1	0	0	9	0	0	0	0	0	5	5	0	0	0	11	0	0
Epiphanidae																
Proalides tentaculatus	35	3	29	0	0	0	13	14	8	9	3	0	12	10	0	0

Appendix 1 continued.

	Trip 1					Trip 2						Trip 3				
	Rushy Island	Ewe Island	Godfrey's Landing	Tauwitchere	Goolwa downstream	Rushy Island	Ewe Island	Godfrey's Landing	Tauwitchere	Goolwa downstream	Goolwa upstream	Rushy Island	Ewe Island	Godfrey's Landing	Tauwitchere	Goolwa downstream
Lecanidae																
Lecane cf. *luna*	0	0	0	0	0	0	0	7	0	0	0	0	0	0	0	0
Lecane sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Notommatidae																
Cephalodella sp. 1	0	0	9	0	0	0	0	0	0	0	0	0	0	0	0	0
Synchaetae																
Synchaeta cf. *oblonga*	0	0	0	0	0	0	0	0	0	0	8	0	0	0	0	0
Synchaeta cf. *triophthalma*	15	64	45	15	56	42	18	0	0	5	0	0	29	30	0	0
Synchaeta cf. *vortax*	0	0	0	0	0	0	0	14	0	0	0	0	6	0	0	0
Testudinellidae																
cf. *Pompholyx complanata*	0	0	0	0	0	8	0	0	0	0	3	0	0	0	0	0
Trichocercidae																
Trichocerca sp 1	18	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Trichocerca sp 2	0	0	0	0	0	4	0	0	0	0	0	0	0	0	0	0
Trichocerca sp 3	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Trichocerca cf. *rattus carinata*	0	0	0	0	0	0	0	0	8	0	0	0	0	0	0	0
Copepoda																
All nauplii	29	27	9	51	16	41	58	50	58	2	1	100	28	21	70	80
Calanoida																
Calanoid (adult + sub-adult)	1	0	0	0	0	0	0	7	0	0	0	0	11	11	0	0

Appendix 1 continued.

	Trip 1					Trip 2						Trip 3				
	Rushy Island	**Ewe Island**	**Godfrey's Landing**	**Tauwitchere**	**Goolwa downstream**	**Rushy Island**	**Ewe Island**	**Godfrey's Landing**	**Tauwitchere**	**Goolwa downstream**	**Goolwa upstream**	**Rushy Island**	**Ewe Island**	**Godfrey's Landing**	**Tauwitchere**	**Goolwa downstream**
Cyclopoida																
Cyclopoid (adult + sub-adult)	0	2	0	0	0	0	0	7	0	3	0	0	3	0	0	0
Harpacticoida																
Harpacticoida (adult + sub-adult)	0	0	0	0	0	0	3	0	0	0	0	0	3	0	30	0
Cladocera																
Bosminidae																
Bosmina meridionalis	0	0	0	6	0	0	0	0	0	10	4	0	0	0	0	0
Daphniidae																
Daphnia carinata	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
Ilyocryptidae																
Ilyocryptus sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	5	0	0
Ostracoda																
Adult	1	0	0	0	0	0	0	0	0	0	0	0	4	0	0	0
Juvenille	0	0	0	0	0	0	8	0	2	0	1	0	0	10	0	0
Decapoda																
Crab zoea	0	0	0	0	4	0	0	0	0	0	0	0	3	0	0	10



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