

Inland Waters and Catchment Ecology

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Diet and trophic characteristics of mulloway (*Argyrosomus japonicus*), congolli (*Pseudaphritis urvillii*) and Australian salmon (*Arripis truttaceus* and *A. trutta*) in the Coorong



George Giatas and Qifeng Ye

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EXECUTIVE SUMMARY

Dietary and food-web structure studies undertaken in the Coorong over the last decade have greatly improved our knowledge of feeding habits and trophic interactions of key fish species within the Coorong. However, most of our knowledge is derived from a prolonged drought (2003–2010), which was characterised by little or no freshwater inflow. Freshwater inflow influences productivity and the abundance, and distribution, of estuarine biota and thus, plays an integral role in determining diet and food-web structure. Knowledge of fish diet and food-web structure during periods of freshwater inflow remains limited.

In the current study, we assessed diet and trophic characteristics (e.g. trophic level) of mulloway (*Argyrosomus japonicus*), congolli (*Pseudaphritis urvillii*) and Australian salmon (*Arripis truttaceus* and *A. trutta*) in the Coorong in 2013-14 during a moderate inflow year, which followed three consecutive years of high freshwater inflows. Diet assessment was based on stomach-content analysis of fish sampled from the Murray Estuary and North Lagoon regions from November 2013 to March 2014. Stomach-content analysis was complemented by an allied study (Johnson 2014) which provided additional information on the general trophic structure of the Coorong food-web using stable isotope analysis (SIA). Key findings are summarised below:

Stomach-content analysis

Mulloway

- Mulloway demonstrated a pronounced ontogenetic shift in diet, but dominant prey items differed substantially for fish collected from the Murray Estuary and North Lagoon regions, which were characterised by different salinities.
- In the Murray Estuary, a progression in total length (TL) from 174–399 mm to 400–699 mm was associated with replacement of the small-bodied clupeid, sandy sprat (*Hyperlophus vittatus*), by prey such as bony herring (*Nematalosa erebi*) and gobies. For the North Lagoon, small crustaceans (mysid shrimp and amphipods) and sandy sprat were replaced by a crab (*Paragrapsus gaimardii*) and congolli with progression in length between the sizes above. Mulloway ≥ 700 mm TL principally preyed upon large yellow-eye mullet (*Aldrichetta forsteri*) in both regions.

- The ontogenetic shift in diet observed in mulloway was likely driven by increased gape size, increased mobility and decreased intraspecific predation pressure with increasing fish size.
- Differences in diet between regions were driven by greater prevalence of freshwater and estuarine fish species (e.g. sandy sprat, bony herring and gobies) in the Murray Estuary and salt-tolerant fish species (e.g. congolli and smallmouthed hardyhead) and crustaceans in the North Lagoon.

Congolli

- Congolli mainly fed on epi-benthic crustaceans and infaunal polychaetes. Diet from fish caught at the freshwater sites in the Lower Lakes was dominated by the freshwater atyid shrimp, *Paratya australiensis*, and contained higher proportions of fish and insects (e.g. corixids, chironomids, odonates) compared to estuarine and marine sites in the Coorong, where the diet was principally comprised of amphipods and polychaetes.
- In the Coorong, diet differed between size classes of congolli; small congolli had greater proportions of small gammaridean amphipods and capitellid polychaetes, while the diet of larger congolli comprised greater proportions of teleosts and large amphipods from the genus *Melita*.
- Congolli demonstrated a clear transition in diet across sites along an increasing salinity gradient; there was a decline in the prevalence of small gammaridean amphipods in the diet with increasing salinity. At the most saline site sampled (Noonameena, mean 54.9 ppt), capitellid polychaetes and large amphipods from the genus *Melita* became the most abundant prey in small and large congolli diets, respectively. This transition in diet appeared to be determined by relative abundances of prey whose distribution may be driven by salinity preferences or sediment characteristics (e.g. grain size and organic matter content).

Australian salmon

- Juvenile Australian salmon were mainly piscivorous, with the contribution of crustaceans (e.g. amphipods and mysid shrimp) in diet decreasing with increasing fish size.
- Sandy sprat and smallmouthed hardyhead (*Atherinosoma microstoma*) were the most important teleost prey items in the diet of >100 mm members of the western species.

SIA

Diet estimation from SIA generally provided good support for key diet items found in the stomachs of the three target species. Trophic position estimation was also consistent with known feeding modes of each species. While an understanding of the general feeding modes (i.e. omnivory, carnivory, piscivory, etc.) of various fish species was gained, results highlight that this technique should not be used in isolation to predict/estimate diet without the accompanying use of stomach-content analysis or at least some diet literature as key prey items may have similar isotopic compositions to other items that weren't consumed.

Comparisons of 2013-14 findings to food-web studies conducted in the Coorong during the drought indicated some differences in trophic structure and diet of mulloway. Most notable was a difference in the diet of mulloway, with increased proportions of sandy sprat and bony herring during 2013-14, relative to drought. A general shift in $\delta^{13}\text{C}$ signatures of teleost fishes was evident, from typically enriched $\delta^{13}\text{C}$ ($\sim -16\text{‰}$ to -10‰) during the drought to depleted $\delta^{13}\text{C}$ ($\sim -20\text{‰}$ to -16‰) in 2013-14. This may suggest greater support of the trophic-web by freshwater-derived organic carbon in 2013-14, relative to the drought, or a shift in the proportion of benthic production (benthic micro- and macroalgae, and detritus-based) verse pelagic production (phytoplankton-based) within the Coorong following moderate-high freshwater inflows. In order to make a more detailed assessment of changes in the Coorong food-web structure in the context of hydrological variability, quantitative assessment would be required considering the abundance of prey and predators, and their trophic position in the food-web.

GENERAL INTRODUCTION

Ecological patterns and processes in estuaries are determined by a range of abiotic (e.g. hydrology) and biotic factors (e.g. trophic interactions), and these factors may interact. For instance, changes in freshwater inflow to estuaries can indirectly modify trophic interactions through changes in nutrient input and thus, productivity (Allanson and Read 1995; Nedwell *et al.* 1999), and by influencing the abundance, composition, and distribution of biotic assemblages via salinity changes (Drinkwater and Frank 1994; Alber 2002). Understanding diet requirements of key species (e.g. fish) and their trophic interactions in estuaries, particularly in the context of hydrology, underpins appropriate management of ecosystems.

Stomach content analysis is a simple and widely adopted method for assessing fish diet and trophic interactions (Hynes 1950; Hyslop 1980). Nonetheless, the capacity of this method to describe the diet of fishes is restricted as the analysis may reflect only recently consumed prey items or those that are more resistant to decomposition. Recently, stomach-content analysis has been partnered with novel approaches such as stable isotope analysis for assessing estuarine fish diet or understanding trophic structure in food-webs (Hadwen *et al.* 2007; Winemiller *et al.* 2007). Stable isotope analyses are complementary to stomach-content analyses as they integrate diet of fish over a considerably greater period (weeks to months), and thus, may provide a more detailed indication of fish diet and in part address the aforementioned limitations of stomach-content analysis when used in isolation (Hadwen *et al.* 2007).

Stable isotope analyses in food-web studies typically involve the use of ratios of isotopic carbon ($\delta^{13}\text{C} = {}^{13}\text{C}:{}^{12}\text{C}$) and nitrogen ($\delta^{15}\text{N} = {}^{15}\text{N}:{}^{14}\text{N}$) from tissues of consumers and prey to provide two different types of information. Firstly, $\delta^{15}\text{N}$ tends to become more enriched ($\sim 3\text{--}4\text{‰}$ per trophic level) in a predictable fashion up the food chain (DeNiro and Epstein 1978; Peterson and Fry 1987) due to the loss of the lighter nitrogen isotope (N^{14}) and may therefore be used to predict the trophic position and feeding mode of consumers. While in contrast, $\delta^{13}\text{C}$ varies at the base of the food-web depending on how organic matter is produced (e.g. C3 vs. C4 plants), but tends to remain similar ($<1\text{‰}$) up the food chain (DeNiro and Epstein 1978; Peterson and Fry 1987) and thus, provides an indication of a trophic pathway. The combined use of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ can therefore provide an understanding of the trophic structure of estuarine food-webs and assist in estimating fish diet.

The Coorong is an estuarine–lagoon system that is located at the terminus of the River Murray, South Australia and considered a wetland of international importance under the *Ramsar Convention* due to its ecological significance (Phillips and Muller 2006). The system supports a range of biota including a diverse fish assemblage, characterised by a spatio-temporally variable assemblage of freshwater, catadromous, anadromous, marine and estuarine fishes. Freshwater inflow to the Coorong has been variable over the last decade and was characterised by hydrological extremes. From 2003–2010, drought and water abstraction in the Murray–Darling Basin were accompanied by low inflows to the Coorong and marine–hypermarine salinities, whilst 2010–2013 was characterised by high inflows and brackish–hypermarine salinities.

Dietary and food-web structure studies undertaken in the Coorong over the last decade (Lamontagne *et al.* 2007; Geddes and Francis 2008; Deegan *et al.* 2010; Giatas 2012; Earl 2014) have greatly improved our knowledge of dietary patterns and trophic interactions of species within the Coorong, particularly fish. Nevertheless, knowledge of the diet of several species, including those of commercial and conservation significance remains limited. Furthermore, the majority of the aforementioned studies were conducted during the drought period when freshwater inflows were low or absent. Fish diet and trophic structure is likely to vary with hydrology (e.g. no inflow vs. high inflow) given that variability in freshwater inflow has a great influence on the diversity, distribution and abundance of various biota in the Coorong, including fish (Livore *et al.* 2013) and benthic macroinvertebrate (Dittmann *et al.* 2013).

The primary objective of this study was to investigate the diet of three fish species in 2013–14 during a moderate inflow year (~1,830 GL) in the Coorong, which followed three consecutive years of high inflows (>5,200 GL/year). These species included a commercially and recreationally important species (mulloway, *Argyrosomus japonicus*), a conservationally significant catadromous species (congolli, *Pseudaphritis urvillii*) and an abundant marine species (Australian salmon, *Arripis truttaceus*). The diets of the latter two species have not been documented in the Coorong. A secondary objective was to provide contemporary information on the trophic structure of the Coorong, focusing on the three species under investigation. Specific aims were to:

- Assess the diet of mulloway, Australian salmon, and congolli in the Coorong using stomach-content analysis;

- Review the findings of an allied stable isotope analysis study (Johnson 2014) to provide information on the contemporary trophic structure of the Coorong and compare findings from stomach-content analysis with stable isotope analysis.
- Compare fish diet results collected in the current project with that presented by other authors from the drought (2003–2010) in the Coorong (Geddes and Francis 2008; Deegan *et al.* 2010).

This report is divided into three parts: 1) detailed presentation of stomach-content analysis; 2) review of findings and interpretation of Coorong food-web structure from stable isotope analysis (Johnson 2014); and 3) synthesis of findings from the current study and comparison to similar studies undertaken during the drought. The following Part (Part 1) presents the findings of stomach-content analysis for each species separately. Each species section is prefaced by a brief review of literature on its general ecology and diet, along with species-specific hypotheses and methods.

PART 1 STOMACH-CONTENT ANALYSIS

1.1 Mulloway

1.1.1 Biology and ecology

Mulloway is a large (>1500 mm), long-lived (41 years) sciaenid (Silberschneider and Gray 2008; Ferguson and Ward 2011) that occurs in estuarine and coastal waters of Australia, South Africa, India, Pakistan, China, Korea and Japan (Gomon *et al.* 2008). Estuaries act as important recruitment habitats for juvenile mulloway and spawning, and recruitment, of this species is influenced by the magnitude of freshwater discharge to estuaries (Hall 1984; Griffiths 1996; Ferguson *et al.* 2008). Size-related differences in estuarine habitat-use have been recorded in mulloway; small juvenile fish (<200 mm) occur in upper reaches (Gray and McDowall 1993; Griffiths 1996), while larger juveniles and sub-adults (>400 mm) are generally more abundant in lower reaches of estuaries (Griffiths 1996). Juvenile and sub-adult mulloway are further spatially segregated from adult fish (~>800 mm), which are found along the ocean beaches and bays of the nearshore marine environment (Griffiths 1996; Ferguson and Ward 2011).

Mulloway is a large predator that has been described as a 'benthic carnivore'; however, this species is capable of feeding throughout the water column (Kailola *et al.* 1993). Mulloway diet has been investigated over different geographical areas including South Australia (SA) (Hall 1986; Geddes and Francis 2008; Deegan *et al.* 2010), New South Wales (NSW) (Taylor *et al.* 2006a) and South Africa (Griffiths 1997). Although prey species may differ between these areas, prey groups and ontogenetic shifts in diet appear similar. Mysid shrimps have been identified as one of the most important food item for small juveniles (<150 mm), followed by a general shift in diet to small teleosts and then larger teleosts and cephalopods with increasing mulloway size (Griffiths 1997; Taylor *et al.* 2006a). Larger crustaceans (e.g. penaeid prawns) have also been recognised as important prey for intermediate sizes (200–500 mm) in both Australia (Taylor *et al.* 2006a) and South Africa (Griffiths 1997). There has been no detailed dietary assessment for the species in estuarine habitats of SA; however, some information is available from a number of small-scale studies conducted in the Coorong (Hall 1986; Geddes and Francis 2008; Deegan *et al.* 2010).

Mulloway plays an important ecological role as an apex predator in the Coorong aquatic food-web (Deegan *et al.* 2010). As well as being ecologically important in the system, mulloway from

the Coorong is of high economic and recreational significance to SA. The species made up 4.8% of total commercially harvested finfish biomass (68 tonnes) in the Lakes and Coorong Fishery in 2013-14 (Earl 2015) and is worth up to \$600,000 annually (Ferguson and Ward 2011). Based on recreational fishing surveys, approximately 44% of mulloway (30,049 individuals) in the State are caught from the Coorong (Jones 2009).

1.1.2 Species-specific aims and hypotheses

Aims:

1. Assess mulloway diet over a broad size range within the Coorong during 2013-14 (moderate inflow);
2. Investigate ontogenetic shifts in diet with mulloway size; and
3. Determine if diet is different along the salinity gradient of the Coorong.

Hypotheses:

1. Diet will differ between different size classes ($n = 3$) of mulloway; and
2. There will be a difference in mulloway diet between two regions that were characterised by different salinities: 1) Murray Estuary; and 2) North Lagoon of the Coorong. Region was selected as the appropriate spatial scale due to the high mobility of mulloway (Taylor *et al.* 2006b; Naesje *et al.* 2012) and patchiness of samples per site (Appendix 1).

1.1.3 Methods

Study area

This study was conducted in the Lower Lakes and Coorong, SA, located at the terminal end of the River Murray (Figure 1). Lake Alexandrina and Albert (i.e. the Lower Lakes) are large, shallow lakes (<3 m), which receive river flows at the northeast corner of Lake Alexandrina. The Coorong is an estuarine–lagoonal system that can be divided into three geographical regions: the Murray Estuary, North Lagoon and South Lagoon based on distinct physical features and salinity properties, generally with increasing salinity from the barrages into the South Lagoon. The Coorong receives freshwater inflows through the barrages at the Lake Alexandrina–Coorong interface and a small creek system (Salt Creek) at the southern end of the South Lagoon. The Coorong connects to the Southern Ocean through the Murray Mouth.

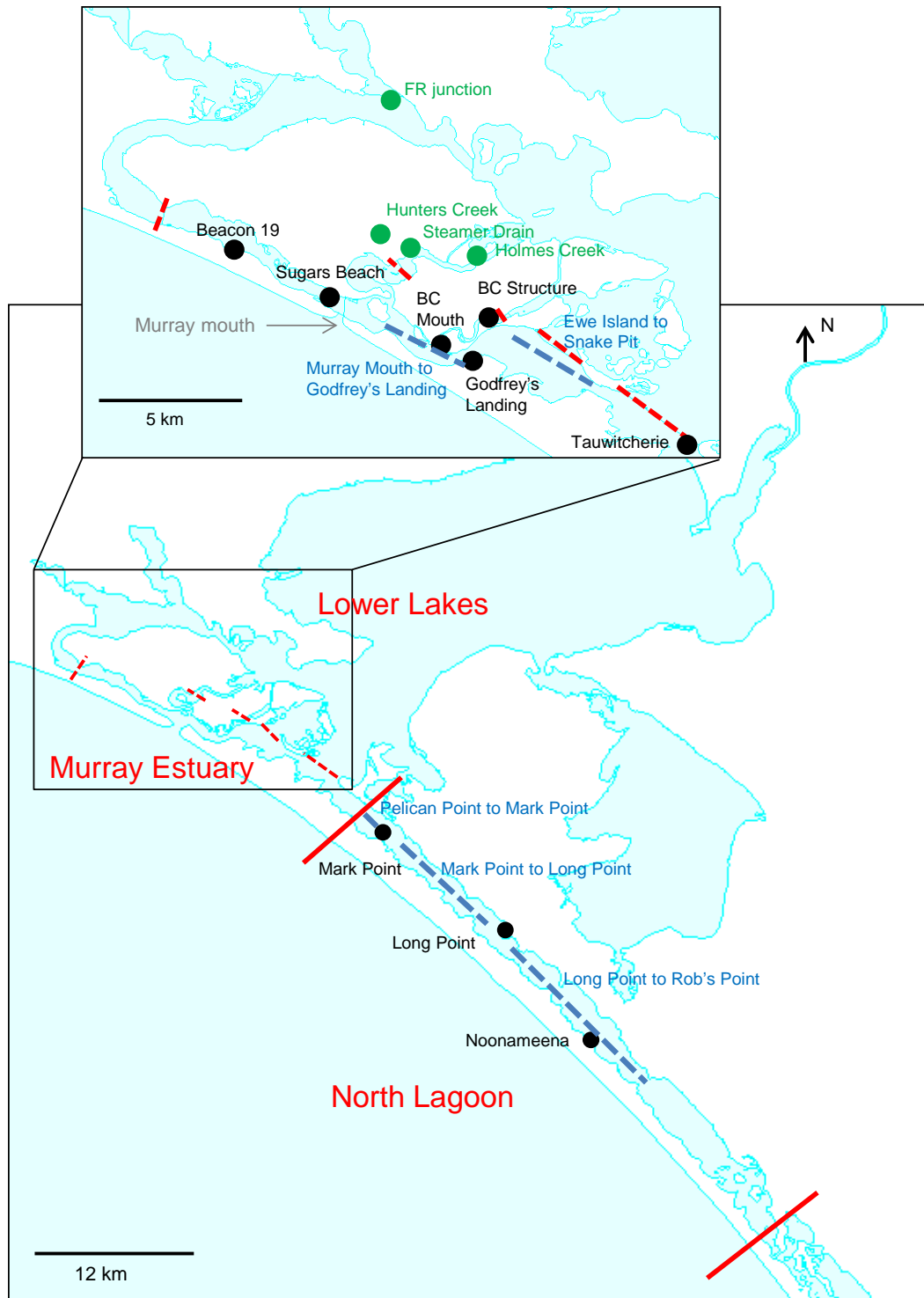


Figure 1. Map of sampling sites in the Coorong and Lower Lakes. Geomorphologic regions are in red text. SARDI monitoring sites are indicated by black points and commercial fishermen sites are shown with dashed blue lines. Green sites are those Lower Lakes sites where congolli were collected (see Section 1.2.3). Solid red lines represent boundaries of the regions. Dashed red lines represent barrages, which divide the Murray Estuary and Lower Lakes regions. Refer to Appendix 2 for GPS coordinates and sampling methods. Salinities for sampling sites can be viewed in Figure 2.

Fish sampling

Fish samples (i.e. mulloway, Australian salmon and congolli) were collected bimonthly (November 2013, January 2014 and March 2014) through fish monitoring projects undertaken by SARDI, using a combination of multi-panel monofilament gill nets (five 9 m panels: 38, 50, 75, 115 and 155 mm stretched mesh) and seine netting (61 m long with 29 m wing length and 22 mm mesh x 3 m bund length with 8 mm mesh). Large (>~550 mm) mulloway were obtained from commercial fishers using large mesh gill nets (~115–150 mm mesh).

Sampling occurred in two Coorong regions: Murray Estuary and North Lagoon (Figure 1). Sampling was conducted at various sites within each region and fish samples were collected opportunistically. Sampling sites and associated sampling methods can be viewed in Appendix 2. Mean salinities across sampling months ranged from 5.8–29.1 and 34.9–67.1 ppt for the Murray Estuary and North Lagoon, respectively (Figure 2). After capture, fish collected via SARDI research sampling were placed into an ice slurry until processing.

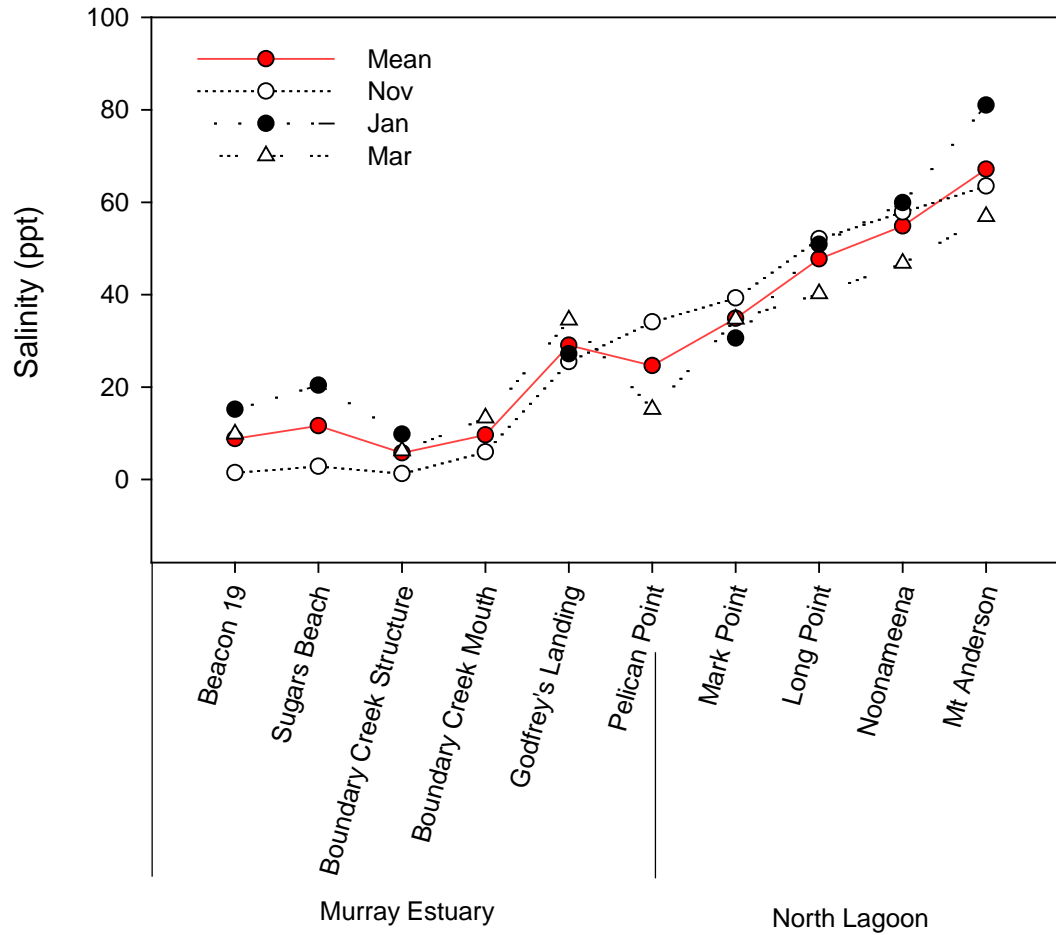


Figure 2. Mid-water salinities of sampling sites in the Coorong during the different months (data from Ye *et al.* 2015). Pelican Point represents the border of the Murray Estuary and North Lagoon regions. Mean salinities of the regions were 5.8–29.1 and 34.9–67.1 ppt for the Murray Estuary and North Lagoon respectively. Note that Mt Anderson and Rob's Point (commercial fisher site, Figure 1) are located close to each other.

Fish processing

Fish were measured for length (to the nearest mm, hereafter total length (TL) unless specified) and weight (to 0.01 g) before being dissected and having their stomach (and oesophagus) contents removed. Stomach-contents were preserved in 70% ethanol or frozen in the case of large prey items (i.e. >150 mm) and transported to the laboratory. Total fish weight was not recorded for samples obtained from commercial fishers.

Stomach-content analysis

Individual items in stomachs were identified to the lowest taxonomic level possible macroscopically or under a dissecting microscope (x 6.5–40), enumerated and measured for wet weight (nearest 0.01 g). Parasites (nematodes and acanthocephalans) found in stomachs were not included in any analysis as they were not considered to be diet. For decomposed teleosts, otoliths (i.e. morphology and size) and digestive tract features were used to assist in identification. Teleosts that were too decomposed for identification (e.g. only skeletons and flesh) were grouped under the category ‘unidentified teleosts’. Food categories were calculated quantitatively by number and weight. The percentage frequency of occurrence (%F), percentage by total number (%N) and total weight (%W) were calculated for different prey items following the equations below (*sensu* Hyslop 1980).

$$\%F = \frac{\text{number of stomachs containing food item}}{\text{total number of stomachs}} \times 100\%$$

$$\%W = \frac{\text{weight of food item}}{\text{total weight of all food items}} \times 100\%$$

$$\%N = \frac{\text{number of food item}}{\text{total number of all food items}} \times 100\%$$

A modified index of relative importance (IRI) (*sensu* Taylor *et al.* 2006a) was calculated to represent the importance of prey items to the consumer. The IRI values were expressed as a percentage (%IRI) per group for simpler interpretation of results. From herein, ‘importance’ of prey items in diet refers to the interpretation of the IRI, i.e. those items with high IRI values are important.

$$IRI = (\%N + \%W) \times \%F$$

Size separation

Samples were split into three size classes to investigate ontogenetic shifts in diet: 1) <400, 2) 400–700, and 3) >700 mm (Table 1). Size class selection was based on literature from South African and NSW estuaries where ontogenetic shifts were observed for mulloway (Griffiths 1997; Taylor *et al.* 2006a).

Table 1. Mean (\bar{x}) mulloway total length (mm) \pm standard deviation (S.D.) per size class for the Murray Estuary (ME) and the North Lagoon (NL) of the Coorong. The <400 mm size class ranged from 174–399 mm and the large size class ranged from 701–945 mm.

Size class (mm)	ME			NL		
	<i>n</i>	\bar{x}	S.D.	<i>n</i>	\bar{x}	S.D.
<400	41	296.0 \pm	52.1	55	286.4 \pm	41.9
400-700	53	550.2 \pm	81.1	76	556.6 \pm	60.8
>700	26	814.7 \pm	64.8	14	789.6 \pm	75.1
Total	120			145		

Statistical analysis

Prior to analyses, all gobies and nereid polychaetes were grouped by their respective families, while all other prey items were analysed as identified. Diet composition data were standardised by total prey weight (i.e. prey item weight was divided by the total weight in that sample, across all prey items) to remove the effect of increased stomach capacity with mulloway length. Samples that contained only unidentified teleosts ($n = 12$) were removed from the analysis to reduce the influence of unidentified prey (value of 1 after standardising) on dissimilarity between factors.

To test for differences between size classes ($n = 3$) and regions ($n = 2$), data were analysed using a two-factor (size, region) multivariate permutational analysis of variance (PERMANOVA) based on Bray-Curtis dissimilarity matrices (dummy variable = 1), in the software program PRIMER v. 6.1.12 and PERMANOVA+ (Clarke and Gorley 2006; Anderson *et al.* 2008). Data were arcsine square-root transformed prior to analyses as the data represented proportions. Significance was set at $p = 0.05$ and p -values were obtained using 9,999 permutations of residuals under a reduced model. When significant differences occurred, PERMANOVA pairwise comparisons were undertaken. To allow for multiple comparisons between regions and sizes, a false discovery rate (FDR) procedure (B–Y method correction) was adopted ($\alpha = \sum_{i=1}^n (1/i)$; e.g. for $n_{comparisons} = 6$, B–Y method $\alpha = 0.05 / (1/1 + 1/2 + 1/3 + \dots + 1/6) = 0.020$) (Benjamini and Yekutieli 2001; Narum 2006). Two-dimensional multi-dimensional scaling (MDS) was used to graphically represent similarities in diet composition by size and region. Where significant differences were detected for PERMANOVA pairwise comparisons between sizes and regions, the contribution of different prey items to the dissimilarity in diet composition was assessed using similarity percentage (SIMPER) analysis (Clarke 1993). A 60% cumulative contribution cut-off was applied.

1.1.4 Results

General diet

Stomach contents from 405 mulloway were examined, of which 140 were empty (34.6%) and excluded from any further analysis (Table 1). Crustaceans were the most important prey group for small mulloway (<400 mm) as indicated by IRI (62.4%), but decreased in importance with increasing size and were replaced by teleosts as the most important mulloway diet group (Table 2). Whilst crustaceans were important dietary items for small and medium-sized mulloway, particularly in the North Lagoon (Appendix 3), teleost fish made up the vast majority of diet by weight for all sizes of mulloway (Table 2, regions combined). Polychaetes, insects and macroalgae made up only a very small proportion of the diet by importance, weight and number. A total of thirteen species of teleosts were identified in stomachs of mulloway, with multiple species from families Clupeidae and Gobiidae.

The three most important prey items (combined IRI = 88.4%) for small mulloway (<400 mm) were small invertebrates: mysid shrimp and amphipods, and the small-bodied clupeid, sandy sprat (Table 2). Amphipods, mysid shrimp and sandy sprat were common items, each occurring in approximately half (51%, 50%, 49%, respectively) of all small fish stomachs. Disregarding unidentified teleosts, other important teleosts were juvenile bony herring and smallmouthed hardyhead (*Atherinosoma microstoma*) (Table 2). The shore-crab, *Paragrapsus gaimardii*, was the most important item (IRI = 39.9%) in the diet of medium-sized mulloway (400–700 mm), followed by medium-sized teleosts: congolli, bony herring, unidentified teleosts, yellow-eye mullet (*Aldrichetta forsteri*), smallmouthed hardyhead and gobies (Table 2). Although seasonal differences were not statistically investigated due to imbalanced sample sizes (Appendix 4) for medium-sized mulloway, *Paragrapsus gaimardii* was most abundant in the diet during November. Large mulloway (>700 mm) diet was comprised almost solely of yellow-eye mullet (94.7% by weight, 90.0% occurrence) (Table 2; Appendix 5), with the majority of specimens >230 mm (Appendix 6). Conspecifics (mulloway) contributed 3.6% to total diet by weight and occurred in 5% of fish.

Table 2. Importance of prey items in the stomachs of three different size classes of mulloway as determined by the Index of Relative Importance (IRI, expressed as a percentage) and the percentage contribution of prey items by weight (%W). Regions are combined – refer to Appendix 3 for further breakdown by region. Refer to Appendix 5 for percentage contribution of prey items to total diet by number and frequency. %IRI is calculated using the IRI values from Gobiidae and Nereididae, not from individual prey items under these categories, hence these items do not have %IRI values presented. Major prey categories are in bold. %IRI for each major group is calculated as the sum of prey item under respective groups.

Family (or higher)	Prey items	Size class (mm)					
		<400		400–700		>700	
		%W	%IRI	%W	%IRI	%W	%IRI
Teleostei		75.9	36.9	70.8	56.1	99.4	99.9
Arripidae	<i>Arripis truttaceus</i>			0.4	<0.1		
Atherinidae	<i>Atherinosoma microstoma</i>	11.7	2.3	2.3	3.4		
Clupeidae	<i>Hyperlophus vittatus</i>	21.7	27.1	0.3	2.5		
	<i>Nematalosa erebi</i>	36.6	2.8	10.7	12.7	<0.1	<0.1
	<i>Sardinops sagax</i>			0.4	<0.1		
Engraulidae	<i>Engraulis australis</i>			0.1	<0.1		
Gobiidae		0.1	<0.1	3.4	3.0		
	<i>Afurcagobius tamarensis</i>			2.8			
	<i>Arenigobius bifrenatus</i>			0.4			
	Gobiidae Unid.	0.1		0.2			
Mugilidae	<i>Aldrichetta forsteri</i>			22.8	7.9	94.7	99.6
Percidae	<i>Perca fluviatilis</i>			1.2	0.2		
Pleuronectidae	Pleuronectidae Unid.			0.4	0.1		
Pseudaphritidae	<i>Pseudaphritis urvillii</i>	0.1	<0.1	22.1	16.3		
Retropinnidae	<i>Retropinna semoni</i>			<0.1	0.1		
Sciaenidae	<i>Argyrosomus japonicus</i>			3.2	0.2	3.6	0.2
Teleostei Unid.		5.6	4.7	3.4	9.8	1.0	<0.1
Crustacea (Malacostraca)		22.4	62.4	27.9	43.9	0.6	0.1
Amphipoda	Amphipoda (all)	2.0	28.7	<0.1	1.4		
Decapoda							
Brachyura	Crab unid.	0.8	0.1	<0.1	<0.1		
Grapsidae	<i>Paragrapsus gaimardii</i>	10.3	0.9	27.8	39.9	0.5	<0.1
Hymenosomatidae	<i>Amarinus lacustris</i>	0.5	0.1				
Portunidae	<i>Ovalipes australiensis</i>					0.1	<0.1
Caridea							
(Palaemonidae)	<i>Macrobrachium</i> sp.	0.5	<0.1				
Unid. Decapod (prawn)				<0.1	<0.1		
Mysidacea	Mysidacea (all)	8.3	32.6	<0.1	2.6		
Polychaeta		1.2	0.6	<0.1	<0.1		
Nereididae		1.1	0.6	<0.1	<0.1		
	<i>Australonereis ehlersi</i>	<0.1					
	<i>Simplisetia aequisetis</i>	0.1					
	Nereid Unid.	1.0		<0.1			
Nephtyidae	<i>Aglaophamus australiensis</i>			<0.1	<0.1		
Polychaeta Unid.	Polychaeta Unid.	0.1	<0.1				
Insecta	Lepidoptera (moth)			<0.1	<0.1		
Macroalgae*	Macroalgae Unid.	<0.1	N/A	1.3	N/A		
Unidentified matter*		0.5	N/A				
<i>n</i> =		96		129		40	

* Macroalgae and unidentified matter are not applicable for %IRI.

Effect of fish size and region on diet composition

PERMANOVA indicated that there was a significant interaction between mulloway size and region on diet composition ($p < 0.05$, Table 3). This suggests that differences in diet composition between sizes were not consistent among regions and vice versa. Nevertheless, all pair-wise comparisons revealed that diet composition of different size classes were significantly different from each other in both the Murray Estuary and North Lagoon (B–Y method corrected $\alpha = 0.020$, Table 3). Furthermore, diet composition of small, medium and large size classes of mulloway was significantly different between regions (B–Y method corrected $\alpha = 0.027$, Table 3). The MDS ordination generally demonstrated grouping of samples by size x region (Figure 3), although medium-sized mulloway from the Murray Estuary exhibited a varied diet (Figure 3).

Table 3. PERMANOVA test results for fish size and region effects on diet composition of mulloway from the Murray Estuary (ME) and North Lagoon of the Coorong (NL), with pair-wise comparisons between size classes/regions. Sizes are small = <400, medium = 400–700, large = >700 mm. After B–Y method FDR correction, $\alpha = 0.020$ for comparisons between sizes (six comparisons) and $\alpha = 0.027$ for comparisons between regions (three comparisons). p -values presented in bold are significant comparisons.

Factor	df	Pseudo- F	$p(\text{perm})$	Pairwise comparisons	t	$p(\text{perm})$
Size	2	35.095	<0.001	<i>Between Regions</i>		
Region	1	7.561	<0.001			
Size x Region	2	6.939	<0.001	Small	3.6592	<0.001
Residual	247			Medium	3.2823	<0.001
				Large	2.3402	0.003
				<i>Between Sizes</i>		
				ME		
				Small, Medium	3.0151	<0.001
				Small, Large	8.1427	<0.001
				Medium, Large	5.4589	<0.001
				NL		
				Small, Medium	5.5920	<0.001
				Small, Large	4.4408	<0.001
				Medium, Large	3.3813	<0.001

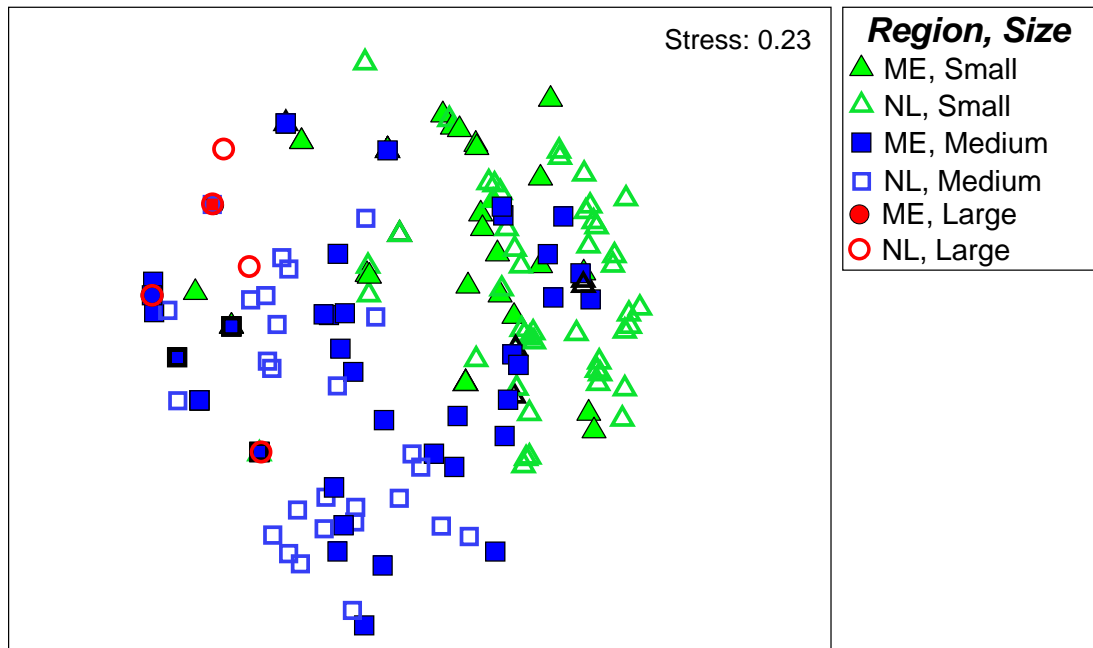


Figure 3. Two dimensional MDS ordination of the dietary compositions (standardised) of three size classes (Small = <400 mm, Medium = 400–700 mm, Large = >700 mm) of mulloway from the Murray Estuary (ME) and North Lagoon of the Coorong (NL). Note that as weight data were standardised, data points may be placed upon one another (i.e. the one Large-ME data point represents 26 Large-ME samples, 9 Large-NL samples and 2 Medium-ME samples).

In the Murray Estuary, differences in diet composition between sizes of mulloway were driven by greater abundances of sandy sprat in the diet of small mulloway; benthic gobies and bony herring in the diet of medium mulloway; and yellow-eye mullet in the diet of large mulloway (SIMPER, Table 4). In the North Lagoon, differences were primarily due to greater abundances of mysid shrimp, sandy sprat and amphipods in the diet of small mulloway; the crab, *Paragrapsus gaimardii*, in the diet of medium mulloway; and yellow-eye mullet in the diet of large mulloway (SIMPER, Table 4).

SIMPER indicated that differences in diet composition between regions for small mulloway were due to greater abundances of sandy sprat in the Murray Estuary, and mysid shrimp and amphipods in the North Lagoon (Table 4). Differences in diet composition between regions for medium mulloway were associated with higher abundances of benthic gobies and bony herring in the Murray Estuary, and the crab, *Paragrapsus gaimardii*, and congolli in the North Lagoon. The average dissimilarity between regions was low (25.3%) for large mulloway (Table 4); yellow-eye mullet was the only prey item found in the stomachs of large mulloway from the Murray Estuary, which was also a major part of the diet for large fish from the North Lagoon

(Table 2; Figure 3). Nevertheless, differences between regions for this size class were significant, owing to the greater abundances of yellow-eye mullet in the Murray Estuary and conspecifics in the North Lagoon (Table 4).

Table 4. Dietary categories selected by SIMPER for typifying (grey boxes) the dietary compositions of three size classes of mulloway. *Indicates that the percentage contribution of a dietary category is greater for the column group. All pair-wise comparisons were significant. The average dissimilarity (%) between groups is presented for each group comparison (unshaded boxes, bold) or average similarity (%) for each group (shaded boxes). ME = Murray Estuary, NL = North Lagoon, S = small (<400 mm), M = medium (400–700 mm), L = large (>700 mm).

Region - Size	ME - S	NL - S	ME - M	NL - M	ME - L	NL - L
ME - S	34.15 <i>H. vittatus</i>					
NL - S	81.21 <i>H. vittatus</i> * Mysidacea Amphipoda	28.96 Mysidacea Amphipoda				
ME - M	88.56 <i>H. vittatus</i> * <i>N. erebi</i> Gobiidae Teleostei Unident.		12.26 Gobiidae <i>N. erebi</i> Teleostei Unident.			
NL - M		96.36 <i>P. gaimardii</i> Mysidacea* <i>P. urvillii</i> <i>H. vittatus</i> * Amphipoda*	91.98 <i>P. gaimardii</i> <i>P. urvillii</i> <i>N. erebi</i> * Gobiidae*	23.47 <i>P. gaimardii</i>		
ME - L	100.0 <i>A. forsteri</i> <i>H. vittatus</i> *		89.80 <i>A. forsteri</i> Gobiidae* <i>N. erebi</i> *		100.00 <i>A. forsteri</i>	
NL - L		99.59 <i>A. forsteri</i> Mysidacea* <i>H. vittatus</i> *		90.69 <i>A. forsteri</i> <i>P. gaimardii</i> *	25.29 <i>A. forsteri</i> * <i>A. japonicus</i>	55.82 <i>A. forsteri</i>

1.1.5 Discussion

Mulloway is known as a benthopelagic carnivore that exhibits changes in diet with ontogeny. In the current study, diet differed between three different size classes of mulloway and between two regions (Murray Estuary and North Lagoon) that were characterised by different salinities. The key prey items identified in the current study and ontogenetic dietary trends are consistent with stomach-content studies conducted elsewhere (Whitfield and Blaber 1978; Marais 1984; Griffiths 1997; Taylor *et al.* 2006a). Marais (1984) demonstrated a similar ontogenetic pattern in four South African estuaries, where small crustaceans (e.g. mysid shrimp) were eaten by small mulloway (<430 mm SL) and replaced by teleost prey with increasing fish size. The small-bodied clupeid, *Gilchristella aesusaria*, was more common in the diet of smaller mulloway, while mugilids were more common in larger mulloway (Marais 1984). Larger crustaceans (i.e. penaeid prawns) have been recognised as important prey for small to intermediate sized (200–500 mm) mulloway in both Australia (Taylor *et al.* 2006a) and South Africa (Griffiths 1997). While large prawns (>80 mm) do not occur in the Coorong, crabs (i.e. shore crab, *Paragrapsus gaimardii*) are abundant and were important in the diet of medium-sized (400–700 mm) mulloway of the North Lagoon.

A number of factors may influence size-related changes in diet of mulloway including increasing gape size, increased mobility and reduced predation pressure with increasing fish size (Elliott *et al.* 2002). Whilst not investigated, increased gape size is the most probable factor influencing the observed transition in diet for mulloway. This is indirectly supported by observations that the largest prey item (i.e. yellow-eye mullet, ~>230 mm TL) dominated the diet of large mulloway only and that the shore crab, *Paragrapsus gaimardii* (max. carapace width 55 mm) was an uncommon dietary item until mulloway were >400 mm and gape allowed consumption of such an item. Taylor *et al.* (2006a) also suggested that increased gape size in mulloway was a key factor, among others, that was facilitating changes in diet with fish size. Increased mobility of larger mulloway (>700 mm) may also be an important factor allowing capture of more mobile prey such as yellow-eye mullet (Elliott *et al.* 2002).

Adult mulloway (>~800 mm) are usually spatially segregated from juveniles and live along ocean beaches; however, some overlap occurs in the Coorong (Ferguson and Ward 2011; Earl and Ward 2014). Findings from this study suggest that small juveniles are prone to cannibalism from larger individuals (>700 mm), particularly since all sizes tend to occupy similar mesohabitats in the Coorong (i.e. deep channels). Conspecific predation has also been documented in South African estuaries (Marais 1984; Griffiths 1997).

Salinity plays a major role in structuring biotic assemblages in estuaries (Sanders *et al.* 1965; Derrick and Kennedy 1997; Kimmerer 2002; Edgar *et al.* 2003; Ysebaert *et al.* 2003). The Coorong is characterised by a strong salinity gradient that increases in a southeast direction from the Murray Estuary region into the South Lagoon of the Coorong. As hypothesised, diet differed between regions characterised by different salinities (i.e. Murray Estuary 5.8–29.1 ppt; North Lagoon 34.9–67.1 ppt) and were primarily driven by greater prevalence of salt-tolerant fish species (e.g. congolli) in the diet of mulloway from the North Lagoon and alternatively, greater abundance of freshwater or estuarine fish species (i.e. sandy sprat, bony herring and gobies) in the diet of mulloway from the Murray Estuary. These findings are consistent with abundances and distributions of these prey species during the sampling period (Ye *et al.* 2015). Crustaceans (e.g. *Paragrapsus gaimardii*) were more prevalent in the diet of mulloway in the North Lagoon. No monitoring data are available for *Paragrapsus gaimardii* in the Coorong, as invertebrate sampling has concentrated on smaller infauna and zooplankton. It is unknown whether the abundance of this species is higher in the North Lagoon, which has been reflected in diet composition, or whether a reduction in prey diversity further into the North Lagoon has resulted in a switch of diet to this species.

Whilst there were spatial differences in diet for all size classes of mulloway, the differences were least dissimilar for large mulloway (>700 mm). Mulloway distribution in the Great Fish River estuary, South Africa, has been closely linked to their primary prey, a clupeid herring, *Gilchristella aestuaria* (Griffiths 1997). Many of the large mulloway (>700 mm) in this study were captured at sites near the border of the two regions (between Ewe Island and Mark Point, Figure 1). Whilst yellow-eye mullet were distributed throughout these two regions (Ye *et al.* 2015), the area between Ewe Island and Mark Point was believed to be where large yellow-eye mullet were in high abundances at the time of sampling (D. Hoad, pers. comm.). Prey assemblage was likely to be similar within this narrow spatial area and thus, may be responsible for the less distinct diet differences between regions. Additionally, the high mobility of larger mulloway compared to smaller mulloway (Hall 1986; Taylor *et al.* 2006b; Naesje *et al.* 2012) may invalidate such a regional comparison, because large mulloway that were caught in one region may have fed in the other region.

Limitations and further research

Season is a factor has been shown to influence diet composition of large sciaenids (Scharf and Schlicht 2000; Taylor *et al.* 2006a). Unevenness in sample sizes for the current study did not

allow for statistical comparison of diet between seasons (Appendix 3). Given that prey assemblages in the Coorong have demonstrated seasonal fluctuations in abundance (Geddes 2005; Dittmann *et al.* 2006; Rolston and Dittmann 2009), mulloway located there may also exhibit seasonal fluctuations in diet. Although not assessed in any detail, *Paragrapsus gaimardii* was recorded in greater abundance in diet in November. This coincided with the typical timing of carapace moulting in this species (G. Hera-Singh, pers. comm.), where crabs have reduced mobility and protection (Ryder *et al.* 1997) and thus, are more susceptible to predation and are a more profitable item for predators (*sensu* Wootton 1992).

Examining the feeding selectivity of mulloway in the Coorong will contribute to the overall understanding of the feeding ecology and dietary requirements of the species. It will be important to consider a wide variety of sampling techniques to assess ambient prey abundances considering that mulloway fed on a combination of fish and invertebrate items, and that not all prey species are well-represented in monitoring datasets.

1.2 Congolli

1.2.1 Biology and ecology

Congolli is a medium-bodied benthic fish species found in coastal rivers of SA, Victoria, Tasmania and southern NSW (Allen *et al.* 2002). Adult females are catadromous and undertake spawning migrations from freshwater to estuarine/marine environments from late autumn to winter (Hortle 1979; Crook *et al.* 2010; Zampatti *et al.* 2011). In contrast, adult males appear to be non-catadromous, residing predominantly in marine or estuarine habitats (Hortle 1979). Size-related sexual dimorphisms are also exhibited by this species, with females growing to a larger size (~350 mm TL) than males (~150 mm TL) (Hortle 1979). Studies using acoustic telemetry (Crook *et al.* 2010; Zampatti *et al.* 2011) and otolith microchemistry (Schmidt *et al.* 2014) provide support for a semelparous life history for congolli. In the Coorong, congolli is capable of spawning over a protracted spawning season (i.e. mid-July to late November); however, spawning intensity is usually greatest in late July/early August (Bice *et al.* 2012). Following spawning, large numbers of juvenile congolli (<60 mm TL) migrate from the marine/estuarine environment to the Lower Lakes between October and January (Bice *et al.* 2012; Bice and Zampatti 2014).

Congolli has been described as an opportunistic carnivore that may exploit a wide range of benthic invertebrates (Hortle and White 1980). The only published study on the diet of congolli was undertaken in south-eastern Tasmania (Hortle and White 1980), with crustaceans, insects (aquatic larvae) and gastropods the primary food items. By volume, the atyid shrimp, *Paratya australiensis*, was the most abundant item in the diet of congolli, followed by mayfly nymphs (*Atalophlebioides* sp.), an estuarine amphipod (*Paracorophium excavatum*) and caddisflies. *Paratya australiensis* became more important in diet with increasing fish length, while chironomids and *Atalophlebioides* sp. declined. In the Coorong, congolli diet was modelled using stable isotope analysis in 2007 during the drought (Deegan *et al.* 2010). Crabs and polychaetes (genera *Capitella* and *Simplisetia*) were estimated to be potential food sources for congolli at Long Point in the North Lagoon of the Coorong (Deegan *et al.* 2010). The diet for this species in the Coorong has not been investigated using stomach-content methods.

Being catadromous, congolli require access between freshwater and marine environments to complete their life cycle. Construction of tidal barrages in the 1940s impeded spawning migrations, likely resulting in a significant population decline from historically high abundances (Evans 1991). Since the late 2000s, a primary conservation focus of the Department of

Environment, Water and Natural Resources (DEWNR) has been to restore populations of congolli by facilitating downstream spawning migrations and upstream juvenile migrations through the construction of fishways on the Murray Barrages (Zampatti *et al.* 2012).

Congolli once supported a component of the commercial fishery prior to population declines in the 1940s (Henry Jones, pers. comm.) and are still caught as bycatch (<150 kg per year) by the Lakes and Coorong Fishery. Congolli is considered to have low commercial value as they are usually not large enough to eat and are traditionally used as bait for other commercial species (Evans 1991). Congolli are likely to play an important ecological role as a food source for large-bodied predatory species such as mulloway (refer to Section 1.1.4) and black bream throughout the Coorong, and as a predator of benthic macroinvertebrates (Hortle and White 1980).

1.2.2 Species-specific aims and hypotheses

Aims:

1. Assess congolli diet within the Coorong during 2013-14 (moderate inflow);
2. Investigate changes in diet with congolli size; and
3. Determine if diet is different along the salinity gradient of the Coorong. In addition, a small sample of congolli was also collected from Lake Alexandrina to investigate congolli diet in a freshwater environment.

Hypotheses:

1. Diet will differ between different size classes ($n = 2$) of congolli; and
2. There will be a difference in congolli diet between sites ($n = 3$) along the salinity gradient of the Coorong.

1.2.3 Methods

Fish sampling

In addition to fish sampling procedures described in Section 1.1.3, congolli were also collected using a large double-winged fyke net (6.0 m long x 2.0 m wide x 1.5 m high with 8.0 m long wings and 6 mm mesh) set at Tauwitcherie (Murray Estuary region, Figure 1) in November 2013 and January 2014 (Appendix 2).

To further investigate the diet of congolli at a range of salinities, a small sample of congolli were collected from a number of sites in Lake Alexandrina (Lower Lakes region, $n = 20$) (Figure 1). These fish were captured using single-wing fyke nets (6 m wing, 0.6 m diameter entry, 8 mm mesh) in November 2013 (Appendix 2). After capture, fish samples were transferred into an ice slurry until processed.

Fish processing

The total length (to the nearest mm) and total weight (to 0.01g) of each individual was recorded. Fish were dissected and the entire alimentary tract was removed and fixed in 10% formalin. Stomach contents were later removed in the laboratory and preserved in 70% ethanol before identification.

Stomach-content analysis

Refer to Section 1.1.3 for methods of prey identification.

For congolli, volume (mm^3), in addition to number (count), was used in substitution of weight to quantitatively calculate food categories due to the small weight of many prey items (<0.01 g). Volume was estimated by squashing contents onto a laminated graph paper with 1 mm^2 squares to a consistent 1 mm height or 5 mm^2 squares to a consistent 5 mm height depending on the size of the item (Hynes 1950; Hyslop 1980). The percentage frequency of occurrence (%F), percentage by total number (%N) (refer to Section 1.1.3) and by total volume (%V) were calculated for prey items (Hyslop 1980).

$$\%V = \frac{\text{volume of food item}}{\text{total volume of all food items}} \times 100\%$$

The index of relative importance (IRI) (Taylor *et al.* 2006a) was calculated to better represent the importance of prey items to the consumer. The IRI values were expressed as a percentage (%IRI) per group for simpler interpretation of results.

$$IRI = (\%N + \%V) \times \%F$$

Size separation

Samples were split into two size classes to investigate changes in diet with size: <80 ; and >80 mm (Table 5). Size class selection was based on the published diet information for this species, where a major shift in diet was observed (Hortle and White 1980).

Table 5. Mean (\bar{x}) total length (mm) \pm standard deviation (S.D.) of congolli from each size class from Tauwitcherie (TAU), Long Point (LP) and Noonameena (NM). The <80 mm size class ranged from 37–79 mm and the >80 mm size class ranged from 80–230 mm.

Size class (mm)	TAU			LP			NM		
	<i>n</i>	\bar{x}	S.D.	<i>n</i>	\bar{x}	S.D.	<i>n</i>	\bar{x}	S.D.
<80	24	48.7 \pm 7.1		39	59.8 \pm 10.7		31	57.7 \pm 13.8	
>80	31	103.8 \pm 17.3		56	119.7 \pm 23.3		37	128.8 \pm 32.6	
Total	55			95			68		

Statistical analysis

Prey items were analysed at a class or order level of identification where appropriate, except for amphipods and polychaetes, due to high occurrence in diet. Polychaetes were analysed at Family level, while amphipods were analysed at Suborder level (i.e. Gammaridea and Senticaudata). Gammaridea was further subdivided into: 1) other gammaridean amphipods (small); and 2) amphipods of the genus *Melita* (large) as sizes of individuals within these two groups are very different and both groups are distinguishable from each other. Large sample numbers were collected from Tauwitcherie, Long Point and Noonameena throughout the sampling months (Table 5). Site spatial distribution along the salinity gradient of the Coorong (Figure 1; Figure 2) was appropriate for investigating potential differences in diet composition, thus only these three sites were included in statistical analyses. Diet composition data were standardised by total prey volume (i.e. prey item volume was divided by the total volume in that sample, across all prey items) to remove the effect of increased stomach capacity with congolli length.

To test for differences between size classes ($n = 2$) and sites ($n = 3$) standardised data were analysed using a two-factor (size, site) multivariate permutational analysis of variance (PERMANOVA) based on Bray–Curtis dissimilarity matrices (dummy variable = 1), in the software program PRIMER v. 6.1.12 and PERMANOVA+ (Clarke and Gorley 2006; Anderson *et al.* 2008). Significance was set at $p = 0.05$ and p -values were obtained using 9999 permutations of residuals under a reduced model. When significant differences occurred, PERMANOVA pairwise comparisons were undertaken. To allow for multiple comparisons between sites and sizes, a false discovery rate (FDR) procedure (B–Y method' correction) was adopted ($\alpha = \sum_{i=1}^n (1/i)$; e.g. for $n_{comparisons} = 6$, B–Y method $\alpha = 0.05 / (1/1 + 1/2 + 1/3 + \dots + 1/6) = 0.020$) (Benjamini and Yekutieli 2001; Narum 2006). Two-dimensional multi-dimensional scaling (MDS) was used to

graphically represent similarities in diet composition by size and site. Where significant differences were detected, pairwise comparisons were undertaken and similarity percentage (SIMPER) analysis (Clarke 1993) was conducted to assess the contribution of different prey items to the dissimilarity in diet composition. A 60% cumulative contribution cut-off was applied.

1.2.4 Results

General diet

The stomach-contents of a total of 324 congolli from the Coorong were analysed. Sixty-seven individuals had empty stomachs (20.7%) and were discarded from any further analysis. Crustaceans were the most important prey group in the diet of small (<80 mm) and large (>80 mm) congolli from the Coorong (IRI = 91.2% and 83.5%, respectively), along with polychaetes. Although teleosts made up a reasonable proportion of large congolli diet (18.2% by volume), this prey category, along with insects and molluscs, did not contribute greatly to overall importance (IRI) in diet for both size classes.

Amphipods were the most important prey item for both size classes from the Coorong (<80 mm, IRI = 87.8%; >80 mm, IRI = 79.8%) followed by nereid polychaetes (<80 mm, IRI = 6.8%; >80 mm, IRI = 15.6%) (Table 6). Diet composition of small congolli was primarily made up of smaller gammaridean amphipods (47.1% by volume) (hereafter referred to as gammaridean amphipods), while large gammaridean amphipods from the genus *Melita*, made a greater contribution to large congolli diet (29.1% by volume). Copepods (IRI = 3.3%) and capitellid polychaetes (IRI = 2.0%) were other important prey items for small congolli, while mysid shrimp (IRI = 3.6%) were important for larger congolli. Teleosts (e.g. smallmouthed hardyhead and gobies) were only eaten by large congolli.

A total of 20 congolli were collected from the Lower Lakes of which seven had empty stomachs (35.0 %). All congolli caught in the Lower Lakes were >80 mm and female. Crustaceans were the most important prey group (IRI = 86.1%), followed by teleosts (IRI = 9.5%) and insects (IRI = 3.7%) (Table 6). The atyid shrimp, *Paratya australiensis*, was the most important prey species in congolli diet (IRI = 69.7%). Other prey items included amphipods, cladocerans (*Daphnia* spp.) and corixids (*Sigara* sp.).

Table 6. Importance of prey items in the stomachs of two different size classes of congolli as determined by the Index of Relative Importance (IRI, expressed as a percentage) and the percentage contribution of prey items by volume (%V). Refer to Appendix 7 for percentage contribution of prey items to total diet by number and frequency. %IRI is calculated using the IRI values from Branchiopoda, Amphipoda, Brachyura, Mysidacea, Copepoda, Chironomidae and Nereididae, not from individual prey items under these categories, hence these items do not have %IRI values presented. Major groups are in bold. %IRI for each major group is calculated as the sum of prey item under respective groups.

Family (or higher)	Prey items	Area		Coorong		Lower Lakes	
		Size class (mm)		<80		>80	
		%V	%IRI	%V	%IRI	%V	%IRI
Teleostei				18.2	0.7	29.6	9.5
Atherinidae	<i>Atherinosoma microstoma</i>			5.2	0.1		
Eleotridae	<i>Philypnodon grandiceps</i>					4.0	0.5
Gobiidae				7.9	0.2		
Poecilidae	<i>Gambusia holbrooki</i>					2.0	0.3
Teleostei Unid.	Teleost Unid.			5.0	0.4	23.6	8.6
	Teleost eggs			<0.1	<0.1		
Crustacea		56.6	91.2	44.8	83.5	67.6	86.1
Branchiopoda				<0.1	<0.1	<0.1	2.9
Cladocera	<i>Daphnia</i> cysts			<0.1		<0.1	<0.1
Malacostraca		53.6		44.8		67.4	
Amphipoda		50.6	87.8	35.2	79.8	0.3	13.0
	Gammaridea						
	Other Gammaridea	47.1		6.0		0.3	
	<i>Melita</i>	3.4		29.1			
	Senticaudata	0.1		<0.1			
	Decapoda	0.4		3.6		67.1	
	Astacoidea (Parastacoidea)					2.7	0.4
	Brachyura	0.4	<0.1	3.6	0.1		
	Crab unid.			3.4			
	Grapsidae						
	<i>Paragrapsus gaimardii</i> (total)	0.3		0.2			
	<i>P. gaimardii</i> (adult)	<0.1		0.2			
	<i>P. gaimardii</i> (post-larvae)	0.2		<0.1			
	<i>P. gaimardii</i> (megalopa)	0.1					
	Hymenosomatidae	0.1					
	<i>Amarinus lacustris</i> (post-larvae)						
	Caridea (Atyidae)					64.5	69.7
	Mysidacea	2.6	0.1	6.1	3.6		
	Mysidacea (adult)	2.6		6.0			
	Mysidacea (post-nauplii stage)			0.1			
	Maxillopoda (Copepoda)	3.0	3.3	<0.1	<0.1		
	Calanoida	0.1		<0.1			
	Cyclopoida	1.3					
	Copepoda Unid.	1.6					
	Ostracoda	<0.1	<0.1				
	Isopoda	0.1	<0.1				
	Crustacea Unid.			0.1	<0.1	0.2	0.1
Insecta				<0.1	<0.1	2.6	3.7
	Diptera			<0.1		0.2	
	Chironomidae			<0.1	<0.1	0.1	1.0
	Chironomidae unid. larvae			<0.1		0.1	

Family (or higher)	Prey items	Area Size class (mm)	Coorong				Lower Lakes	
			<80		>80		>80	
			%V	%IRI	%V	%IRI	%V	%IRI
Chironomidae cont.	Chironomidae unid. pupae						0.1	
Stratiomyidae	Stratiomyidae (larvae)						<0.1	0.1
Unid. Diptera	Unid. Diptera						0.1	0.1
Hemiptera							0.6	
Corixidae	<i>Sigara</i> sp.						0.5	1.3
Fulgoroidea	Fulgoroidea unid.						<0.1	0.1
Hebridae	<i>Hebrus axillaris</i>						<0.1	0.1
Odonata							1.8	
Aeshnidae	Aeshnidae unid. (nymph)						0.5	0.2
Coenagrionidae	<i>Ischnura heterosticta</i> (nymph)						1.2	0.6
Trichoptera	<i>Ecnomus</i> sp.						0.1	0.1
Bivalvia	<i>Arthritica</i>			<0.1	<0.1			
Gastropoda		0.2	<0.1	0.3	<0.1	0.1	0.8	
Hydrobiidae		0.1	<0.1	0.1	<0.1			
Gastropoda Unid.		0.1	<0.1	0.1	<0.1	0.1	0.8	
Polychaeta		43.0	8.7	36.2	15.7			
Capitellidae		13.8	2.0	0.9	0.2			
Nereididae		29.1	6.8	35.3	15.6			
	<i>Australonereis ehlersi</i>	0.5		4.7				
	<i>Simplisetia aequisetis</i>	1.2		14.8				
	Nereid Unid.	27.4		15.8				
Oligochaeta		0.2	<0.1	<0.1	<0.1			
Sipuncula				<0.1	<0.1	<0.1	<0.1	
Plant/Macroalgae*	Plant/Macroalgae Unid.	<0.1	N/A					
Unidentified matter*				0.2	N/A			
Unidentified				0.1	N/A			
<i>n</i> =			121		134		13	

* Macroalgae and unidentified matter are not applicable for %IRI.

Effect of size and site on diet composition in the Coorong

PERMANOVA indicated that there was a significant interaction between congolli size and site on diet composition ($p < 0.05$, Table 7), which suggests that differences in diet composition between sizes were not consistent among sites and vice versa. Nevertheless, pair-wise comparisons revealed significant differences in diet composition between all three sites for each size class (B–Y method corrected $\alpha = 0.020$, Table 7). Furthermore, diet composition differed significantly between sizes at each site (B–Y method corrected $\alpha = 0.027$, Table 7). The MDS ordination demonstrated some grouping of samples by size x site (Figure 4).

Table 7. PERMANOVA test results for fish size and site effects on diet composition of congolli from the Coorong, with pair-wise comparisons between size classes/sites. Sizes are small = <80 and medium = >80 mm. After B–Y method FDR correction, $\alpha = 0.020$ for comparisons between sites (six comparisons) and $\alpha = 0.027$ for comparisons between sizes (three comparisons). p-values presented in bold are significant comparisons. TAU = Tauwitcherie, LP = Long Point and NM = Noonameena.

Factor	df	Pseudo-F	p(perm)	Pairwise comparisons	t	p(perm)
Size	1	18.113	<0.001	<i>Between Sites</i>		
Site	2	27.727	<0.001			
Size x Site	2	4.932	<0.001	a) Small		
Residual	215			TAU, LP	3.5731	<0.001
				TAU, NM	5.2223	<0.001
				LP, NM	3.2846	<0.001
				a) Large		
				TAU, LP	3.7937	<0.001
				TAU, NM	4.2758	<0.001
				LP, NM	4.6505	<0.001
				<i>Between Sizes</i>		
				TAU	3.7200	<0.001
				NM	2.2833	0.002
				LP	3.5372	<0.001

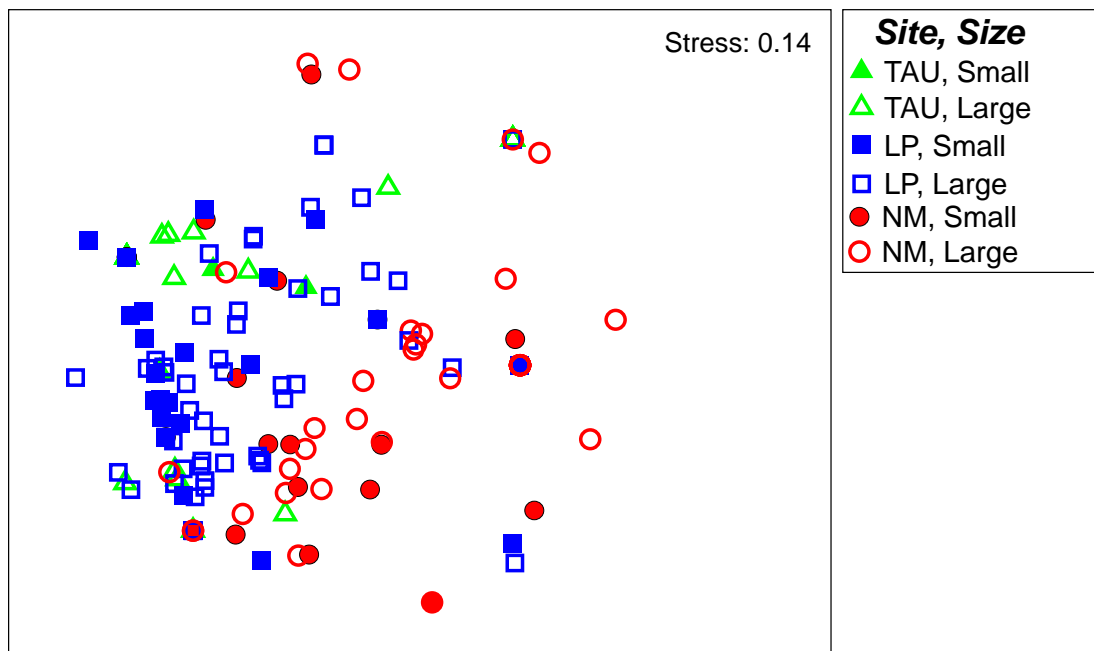


Figure 4. Two dimensional MDS ordination of the dietary compositions (standardised) of two size classes (Small = <80 mm, Large = >80 mm) of congolli at three sites within the Coorong. TAU = Tauwitcherie, LP = Long Point and NM = Noonameena. Note that as volume data were standardised, data points may be placed upon one another.

SIMPER indicated difference in diet composition between size classes at Tauwitcherie was due to greater abundances of gammaridean amphipods in the diet of small congolli and teleost fish in the diet of large congolli (Table 8). Differences in diet composition between size classes at Long Point were driven by higher abundances of gammaridean amphipods in the diet of small congolli and nereid polychaetes in the diet of large congolli (Table 8). Differences between size classes at Noonameena were driven by greater abundances of capitellid and nereid polychaetes in the diet of small congolli, and amphipods from the genus *Melita* in the diet of large congolli (Table 8).

Table 8. Dietary categories selected by SIMPER for typifying (grey boxes) the dietary compositions of two size classes of congolli (unshaded boxes). *Indicates that the percentage contribution of a dietary category is greater for the column group. Significant pair-wise comparisons are presented in bold. The average dissimilarity (%) between groups is presented for each group comparison (unshaded boxes) or average similarity (%) for each group (shaded boxes). TAU = Tauwitcherie, LP = Long Point and NM = Noonameena, S = small (<80 mm), L = large (>80 mm).

Site - Size	TAU - S	LP - S	NM - S	TAU - L	LP - L	NM - L
TAU - S	95.56 Gammaridea					
LP - S	43.47 Gammaridea* Nereididae	44.28 Gammaridea				
NM - S	82.24 Gammaridea* Capitellidae	80.57 Gammaridea* Nereididae* Capitellidae	22.34 Capitellidae <i>Melita</i>			
TAU - L	51.92 Gammaridea* Teleostei			35.62 Gammaridea		
LP - L		70.66 Gammaridea* Nereididae		80.97 Gammaridea* Nereididae Teleostei*	37.28 Nereididae	
NM - L			78.62 <i>Melita</i> Capitellidae* Nereididae*	92.59 <i>Melita</i> Gammaridea* Teleostei*	85.18 <i>Melita</i> Nereididae* Mysidacea*	34.37 <i>Melita</i>

There were transitional changes in diet at sites across the salinity gradient (Figure 5); for both sizes, gammaridean amphipods decreased in importance in diet from Tauwitherie to Noonameena. These observed trends in the %IRI of prey items (Figure 5) were supported by SIMPER (Table 8). Differences in diet composition between sites for small congolli were driven by greater abundances of gammaridean amphipods at Tauwitherie; nereid polychaetes at Long Point; and capitellid polychaetes at Noonameena (Table 8). For large congolli, differences in diet composition between sites were primarily due to greater abundances of gammaridean amphipods and teleosts at Tauwitherie; nereid polychaetes at Long Point; and *Melita* spp. at Noonameena (Table 8). Average dissimilarity in diet composition was greatest between the two furthest sites (i.e. Tauwitherie and Noonameena) for both size classes.

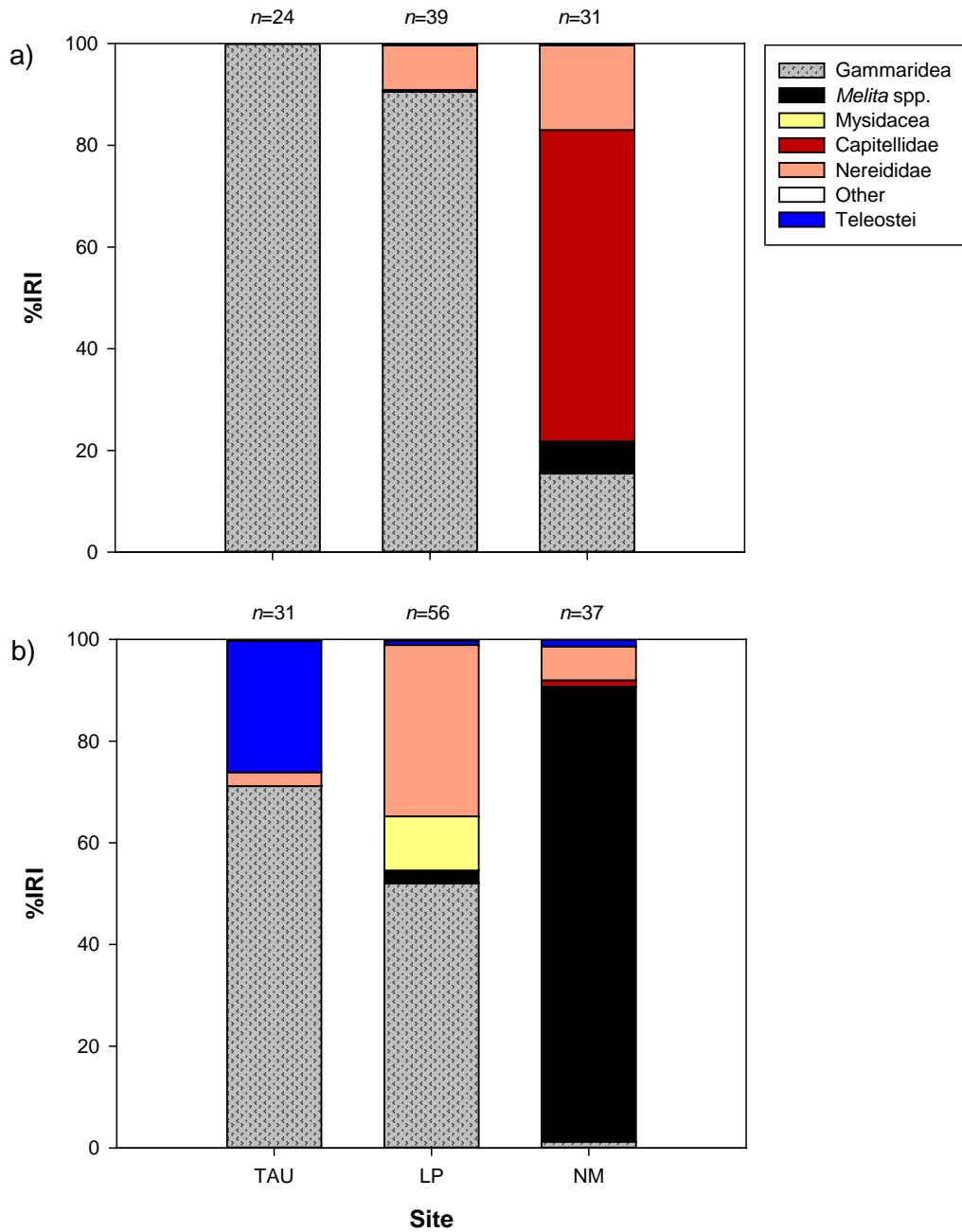


Figure 5. Importance of prey categories (% index of relative importance, IRI) in small (a, <80 mm) and large (b, >80 mm) congolli diet at three sites in the Coorong. Sample numbers are provided at the top of each bar. Other = Cladocera, Senticaudata (Amphipoda), Branchyura, Copepoda, Ostracoda, Isopoda, Oligochaeta, Sipuncula, Mollusca and Insecta. Sites are TAU = Tauwitchee, LP = Long Point and NM = Nooameena.

1.2.5 Discussion

Congolli is an important catadromous fish species with significant ecological and conservation values in the Coorong and Lower Lakes. The current stomach-content study has greatly improved our understanding of the species' feeding habits, especially considering that no diet literature was previously available for congolli in SA. Congolli is a benthic carnivore that is also capable of feeding on prey items in the water column. In the current study, diet differed between two size classes of congolli and between three sites along the Coorong, which had different salinities. Congolli mainly fed on crustaceans and polychaetes; amphipods and nereid polychaetes were the most important prey items for both sizes of congolli in the Coorong, while atyid shrimp (*Paratya australiensis*) were important for large congolli in the Lower Lakes.

The only published study on congolli diet (Hortle and White 1980) was undertaken in south-eastern Tasmania. In both the current and previous study, congolli fed on a variety of prey including crustaceans, insects and molluscs. Hortle and White (1980) found that diet composition at the estuarine site of Browns River was dominated by amphipods and an atyid shrimp (*Paratya australiensis*), which was very similar to results of the current study in the Lower Lakes and Coorong. However, polychaetes, which were prominent in the diet of congolli in the Coorong, were absent in the Tasmanian study. Among other factors (e.g. physical habitat), this may be attributed to differences in salinities of the investigations (Tasmania, 0–25 ppt; Coorong, 25–60 ppt) and that polychaetes (e.g. *Simplisetia aequisetis* and *Capitella* spp.) are generally more abundant in estuarine to marine salinities (Derrick and Kennedy 1997; Edgar *et al.* 2003; Ysebaert *et al.* 2003).

Gammaridean amphipods and nereid polychaetes were the most important prey items for both sizes of congolli; however, larger prey items such as teleosts and large gammaridean amphipods from the genus *Melita* were more abundant in large congolli diet and responsible for driving dissimilarity between size classes. Alternatively, small congolli ate higher proportions of smaller gammaridean amphipods and capitellid polychaetes. Although not investigated during this study, the difference in diet between size classes of congolli in the current study could be a reflection of increasing gape size, allowing capture of larger prey items. Both size classes occupy the same mesohabitat (shallow bank habitats) in the Coorong; therefore, the effect of physical habitat influencing diet is considered to be minimal. Hortle and White (1980) assessed changes in diet with size in a freshwater river (North West Bay River); therefore, direct comparisons with the current Coorong study are not appropriate. Nevertheless, Lower Lakes

data support findings by Hurtle and White (1980) where the large shrimp, *Paratya australiensis*, became more prevalent in diet with increasing fish length.

Transitional changes in diet across the salinity gradient from Tauwitchee (mean 24.7 ppt) to Noonameena (mean 54.9 ppt) were observed in the current study. Small congolli diet showed a relatively simple pattern; as salinity increased, there was a progression in diet from amphipods to polychaetes (i.e. capitellids). Derrick and Kennedy (1997) observed a similar feeding pattern along a salinity gradient for another benthic feeder (flatfish, *Trinectes maculatus*) in estuarine rivers of Chesapeake Bay, USA. Results from the current study align well with transitional changes in the Coorong benthic macroinvertebrate assemblage (Dittmann *et al.* 2014b), which suggests feeding in relation to availability of benthic prey items ('non-selective feeding'). For example, subtidal amphipod abundance was high at Pelican Point (close to Tauwitchee) and steadily decreased to Noonameena, while *Capitella* sp. was absent at Tauwitchee and most abundant at Noonameena (Dittmann *et al.* 2014b).

For large congolli, amphipods were the dominant prey item across all sites; however, teleost fish were more abundant in diet at Tauwitchee, while nereid polychaetes were more abundant at Long Point. Also, for large congolli, the amphipod assemblage in diet changed across the salinity gradient from one dominated by small gammaridean amphipods to one that was made up almost solely of one genus, *Melita*. Coorong subtidal macrobenthic data shows a high abundance of the nereid, *Simplisetia aequisetis*, at both Tauwitchee (i.e. Pelican Point) and Long Point, and low abundance at Noonameena (Dittmann *et al.* 2014b). Given that amphipod abundance was lower at Long Point compared to Tauwitchee, the assumption that nereid polychaetes would be more prevalent in diet at Long Point is consistent with stomach-content results. Despite very low observed abundances of amphipods (all species) at Noonameena (Dittmann *et al.* 2014b), the large gammarid, *Melita* spp. was the dominant prey item in large congolli diet. This may suggest that large congolli show a preference for this particular item.

While salinity could be a major factor in determining congolli diet composition, through structuring the prey assemblages of macrobenthic invertebrates (salinity tolerances/preferences), site macrohabitat characteristics (e.g. grain size, organic matter content and macrophyte/algal composition) may also play an important role (Ysebaert *et al.* 2003; Hirst and Kilpatrick 2007). For example, sites with high microalgal biomass or organic matter may support higher abundances of deposit-feeding polychaetes (e.g. capitellids).

Diet composition of large congolli (>80 mm) was dissimilar between the Lower Lakes (freshwater, <1 ppt) and Coorong (estuarine/marine 25–60 ppt). Diet from the freshwater sites was dominated by the freshwater atyid shrimp, *Paratya australiensis*, and contained higher proportions of fish and insects (e.g. corixids, chironomids, odonates) relative to the estuarine/marine sites, where diet was made up principally of amphipods and polychaetes. These results are consistent with general differences in ambient benthic invertebrate prey assemblages between the two environments, i.e. lower proportional abundances of annelids and higher abundances of insects and carid shrimp in the Lake Alexandrina (Corbin *et al.* 2014; Dittmann *et al.* 2014a). Hortle and White (1980) also found that diet varied between habitats (estuarine vs. freshwater) and that amphipods were dominant in the estuarine habitats, while insects were more prevalent in freshwater habitats. However, contrary to our results, *Paratya australiensis* was more abundant in the diet of congolli from the estuary. This discrepancy could be explained by preferences exhibited by this species (particularly adults) for lower salinities and areas of high vegetation (Williams 1977; Walsh and Mitchell 1995), and that the estuarine region in the current study was characterised by moderate salinities (>25 ppt) and low vegetation. Alternatively, reduced connectivity between the Lower Lakes and Coorong (i.e. barrages) may restrict seasonal migrations by the species from freshwater to estuarine environments (Walsh and Mitchell 1995), and its absence from the Coorong (Dittmann *et al.* 2014a).

Limitations and further research

Season is a factor that can potentially influence diet composition of congolli (Hortle and White 1980). In the current study, samples were pooled from different months as sample sizes did not allow for seasonal comparisons (Appendix 5). Congolli are expected to exhibit some form of seasonal variations in diet considering that their main prey items (benthic macroinvertebrates) demonstrate variations in abundance with season in the Coorong (Geddes 2005; Dittmann *et al.* 2006; Rolston and Dittmann 2009).

Acoustic telemetry and genetic studies suggest that congolli is a semelparous, catadromous (females) species that spawns in the marine environment during late winter/early spring (Crook *et al.* 2010; Zampatti *et al.* 2011; Schmidt *et al.* 2014). Following spawning, large numbers of juvenile congolli (<60 mm TL) migrate to the Coorong and Lower Lakes, through the Murray Barrage fishways between October and January (Bice *et al.* 2012; Bice and Zampatti 2014). While most congolli in the current study were captured at Long Point and Noonameena (North

Lagoon region), a number of small congolli were captured at Tauwitchenie, presumably during migration to the Lower Lakes (freshwater environment). It is possible that some of these individuals may have fed outside of the site of capture and even potentially outside of the Coorong. Nonetheless, all prey items that were identified in this study for congolli commonly occur within the Coorong.

It is important to note that this study focused on one habitat component (the Coorong) of the species' life history, although some insight into the diet of large congolli was gained for the Lower Lakes. Future congolli diet research should be targeted towards investigating diet from freshwater habitats (i.e. Lower Lakes and lower River Murray).

1.3 Australian salmon

1.3.1 Biology and ecology

Australian salmon occur in temperate marine shallow water zones and onshore pelagic habitats of Australia and New Zealand (Gomon *et al.* 2008). In Australia, Australian salmon are comprised of a western (*A. truttaceus*) and eastern (*A. trutta*) species; the western species is the only one that occurs in Western Australia (WA) and the eastern species is the only one to occur in NSW (Malcolm 1960). Both species occur together in parts of Victoria and Tasmania, with minor overlap of the eastern species also in the southeastern parts of SA such as the Coorong (D. Fler, unpublished data). The western species can reach an age of approximately 9 years and a fork length (FL) of over 800 mm (Cappo 1987). Similar maximum lengths (780 mm FL) (Hutchins and Swainston 1986) and ages (12 years) (Stewart *et al.* 2011) have been reported for the eastern species in Australia.

The western Australian salmon spawns between February and May along southwestern Australia (Malcolm 1960; Cappo 1987). Eggs, larvae and post-larval fish are transported eastwards by the Leeuwin current and other wind-driven currents and juvenile salmon settle in sheltered gulfs, creeks and estuaries in SA, Victoria and Tasmania (Jones 1999). Young-of-the-year western Australian salmon (~50–65 mm TL) have been collected from July to September in the Port Adelaide River, SA (D. Fler, unpublished data). SA is considered to be the principal nursery area for the western species, where juveniles (<300 mm TL) reside in bays and estuaries before moving away from gulf waters to medium–high energy coastal beaches, rocky coastlines and offshore islands as sub-adult fish (>300 mm TL) (Malcolm 1960). Larger fish (3–6 years of age) from SA then begin to migrate to WA to spawn (Cappo 1987). Breeding by the eastern species takes place along the coast of Victoria and southern NSW between October and March, with eggs and larvae transported via the east Australian current (Nicholls 1972; Stanley and Malcolm 1977). Incidental catches during research sampling suggests that the eastern species settles in SA during early autumn at a similar size (50–65 mm TL) as the western species (D. Fler, unpublished data). Return spawning movements to the eastern coast of Australia are largely undocumented.

Australian salmon are voracious piscivores (Hughes *et al.* 2013). Diet and the feeding ecology of the two species have been previously considered to be different, which is likely influenced by differences between the numbers and morphology of gill rakers (Malcolm 1959). The western species has 25–31 short, stubby gill rakers on the first gill arch and the eastern species had 33–

40 long, fine gill rakers (Malcolm 1959). The longer, finer and larger number of gill rakers for the eastern species is an adaptation for catching smaller prey (i.e. crustaceans) compared to the western species, which is more suited for fish prey (Stanley 1980). More recent studies (post-1980s) on the diet of the two species from a number of States show similarities in diet; pelagic schooling teleosts such as pilchards (*Sardinops* spp.), scad (*Trachurus* spp.), sandy sprat and anchovy (*Engraulis australis*) are the dominant prey items of larger individuals (Cappo 1987; Hoedt and Dimmlich 1994; Stewart *et al.* 2011), and benthic and pelagic crustaceans (amphipods and shrimps) and fish (gobies, atherinids and sandy sprat) are more important in juvenile diets (Robertson 1982; Stewart *et al.* 2011). Dietary information is lacking for Australian salmon in the Coorong.

Juveniles of the western species are abundant in the Coorong (Livore *et al.* 2013) and likely utilise the estuary as a nursery area; exploiting food resources and protection from predators (Malcolm 1960). This species may play an important role as a food source for larger predatory fish species (i.e. mulloway and black bream, *Acanthopagrus butcheri*). Australian salmon are captured in low quantities in small-mesh commercial gill nets targeting yellow-eye mullet in the Coorong (Ferguson 2010), so may be of some economic value to SA Fisheries.

1.3.2 Species-specific aims and hypotheses

Aims:

1. Assess Australian salmon diet within the Coorong during 2013-14 (moderate inflow); and
2. Investigate size-related shifts in diet of Australian salmon.

Hypothesis:

1. There will be a progression change in diet from small items (e.g. small invertebrates) to larger items (e.g. teleosts) with increasing fish size.

1.3.3 Methods

Fish sampling

Refer to fish sampling described in Section 1.1.3.

Fish processing

In addition to the fish processing procedures described in Section 1.2.3, gills from each individual were preserved and gill raker counts on the first gill arch were performed to discern the eastern species from the western as sympatric overlap of the two is known to occur in the study area (D. Flier, unpublished data). While the majority of Australian salmon investigated in this study were of the western species, a number of small juveniles (<100 mm) of the eastern species were collected during March (Appendix 9).

Stomach-content analysis

Refer to stomach-content analysis described in Section 1.2.3.

Statistical analysis

No statistical analyses were performed on Australian salmon diet. There were no regional comparison aims for this species since Australian salmon were only caught within the Murray Estuary region (Figure 1). It was not suitable to compare diet between different months for this species as the progression of size for the same cohort with season (Appendix 9) may have confounded results and differences between months may be reflective of different sizes of fish compared not seasonal variation. Because of this, the comparison between sizes needs to be treated with caution as this may be an influence of monthly variability, and given that only a narrow size range (56–205 mm) of salmon were collected.

1.3.4 Results

General diet

The stomach-contents of a total of 90 Australian salmon were assessed. Twelve individuals had empty stomachs (13.3%) and were discarded from any further analysis. Many individuals were observed with stomachs filled to capacity and prey protruding out of their throats ('gorging' behaviour) (Appendix 10). Juvenile Australian salmon (56–205 mm) had a carnivorous diet comprised mainly of teleosts and crustaceans (Table 9). The contribution of insects, molluscs and polychaetes to the overall diet was negligible.

Many stomach samples were comprised of a large proportion of unidentifiable teleosts (44.9% of fish). Small-bodied sandy sprat (IRI = 20.9%) and smallmouthed hardyhead (IRI = 14.8%) were the most important teleost prey behind unidentified teleosts. Gobies (particularly Tamar

goby, *Afurcagobius tamarensis*) made up a considerable proportion of the diet by weight (19.4% collectively), but were not as common as sandy sprat and smallmouthed hardyhead (Appendix 11). Cyclopoid copepods (IRI = 14.8%), amphipods (IRI = 13.7%) and mysid shrimp (IRI = 4.4%) were the most important crustaceans in the diet of Australian salmon.

Table 9. Importance of prey items in the stomachs of Australian salmon (56–205 mm, $n = 78$) as determined by the Index of Relative Importance (IRI, expressed as a percentage) and the percentage contribution of prey items by weight (%W). Refer to Appendix 11 for percentage contribution of prey items to total diet by number and frequency. Major groups are in bold. %IRI for each major group is calculated as the sum of prey item under respective groups. Western ($n = 58$) and eastern ($n = 20$) Australian salmon are not separated.

Family (or higher)	Prey items	%W	%IRI
Teleostei		90.2	68.3
Atherinidae	<i>Atherinosoma microstoma</i>	34.1	14.8
Clupeidae	<i>Hyperlophus vittatus</i>	16.2	20.9
	<i>Nematalosa erebi</i> (post-larvae)	0.8	0.2
Galaxiidae	<i>Galaxias maculatus</i>	4.0	0.3
Gobiidae	<i>Afurcagobius tamarensis</i>	12.5	2.9
	<i>Arenigobius bifrenatus</i>	2.8	0.1
	<i>Tasmanogobius lasti</i>	0.7	<0.1
	Gobiidae Unid.	3.4	0.9
Mugilidae	<i>Aldrichetta forsteri</i>	4.1	0.2
Teleostei Unid.	Teleostei Unid.	11.6	26.5
Crustacea		4.3	33.0
Malacostraca			
Amphipoda	Amphipoda	2.4	13.7
Decapoda			
Brachyura	Crab unid.	<0.1	<0.1
Caridea			
(Palaemonidae)	<i>Macrobrachium</i> sp.	<0.1	<0.1
Mysidacea	Mysidacea (all)	1.4	4.4
	Mysidacea (adult)	1.4	3.2
	Mysidacea (post-nauplii stage)	<0.1	0.3
Maxillopoda (Copepoda)	Cyclopoida	0.2	14.8
Ostracoda	Ostracoda	<0.1	<0.1
Unid. Crustacea*	Unid. Crustacea (fragments)	0.2	N/A
Insecta		0.1	<0.1
Chironomidae	<i>Tanytarsus</i> sp. pupae	<0.1	<0.1
Corixidae	<i>Sigara</i> sp.	<0.1	<0.1
Notonectidae	<i>Anisops</i> sp.	0.1	<0.1
Mollusca		3.4	0.1
Donacidae	<i>Donax deltoides</i>	3.4	0.1
Hydrobiidae	Hydrobiidae	<0.1	<0.1
Polychaeta	Nereididae Unid.	0.4	0.1
Macroalgae*	Plant Material Unid.	<0.1	N/A
Unidentified matter*	Unidentifiable matter	1.5	N/A

* Macroalgae, unidentified Crustacea (fragments) and unidentified matter are not applicable for %IRI.

Broad ontogenetic/seasonal shifts in diet for *A. truttaceus*

While small invertebrates (copepods, amphipods and mysid shrimp) made up only a small proportion of the diet by weight (4.3%) for the broad size range (56–205 mm) (Table 9), they collectively formed 42.8% of the diet by weight for individuals <100 mm and were very common (e.g. mysid shrimp, 45.2% occurrence). Mysid shrimp only occurred in the stomachs of individuals <100 mm for both species and copepods only occurred in eastern Australian salmon <75 mm (Figure 6). Figure 6 demonstrates a progression shift in diet for the western species from a mix of teleosts and small invertebrates (at mean length, 83.7 mm) in November to a diet that is made up almost solely of teleosts in March (at mean length, 189.1 mm). The importance of teleost species changed with size/month for the western species from bony herring post-larvae in November (at mean length, 83.7 mm) to sandy sprat in January (at mean length, 148.7 mm), and smallmouthed hardyhead during March (at mean length 189.1 mm) (Figure 6).

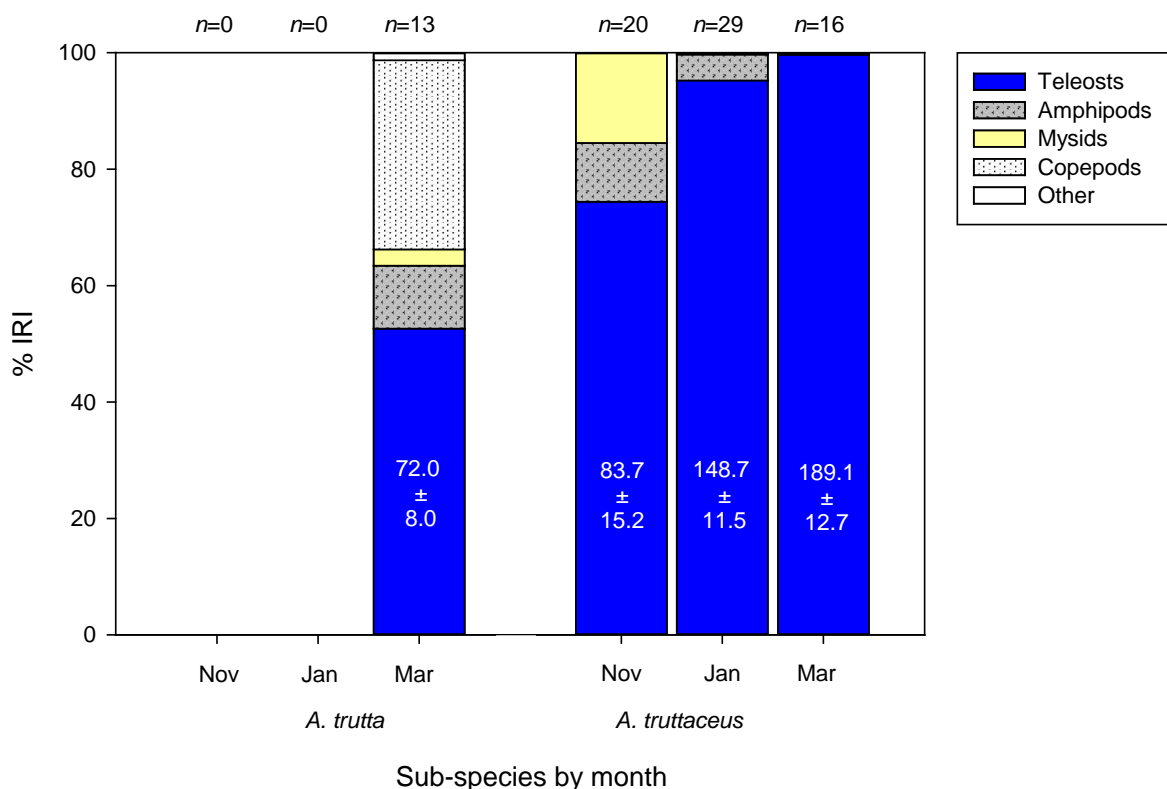


Figure 6. Importance of prey categories (% index of relative importance, IRI) for the two different species of Australian salmon in the Coorong. Mean total length and standard deviations (mm) are given for each species for each month. Sample numbers are provided at the top of each bar. No individuals from the eastern species (*A. trutta*) were collected during November and January. Other = other crustaceans, molluscs, polychaetes and insects.

1.3.5 Discussion

Australian salmon are marine estuarine-opportunists that use estuaries and sheltered bays as nursery areas during their juvenile life stages (Malcolm 1960; Cappel 1987). In the current study, we assessed diet of juvenile Australian salmon (56–205 mm) in the Coorong; the majority of which (74%) belonged to the western species. Teleosts were the main prey category for Australian salmon of all sizes; however, small crustaceans (copepods, amphipods and mysid shrimp) were also important in the diet of small individuals (i.e. <100 mm).

The findings from this study are similar to those conducted on juvenile western Australian salmon in marine embayments of Victoria (Robertson 1982; Hindell *et al.* 2000; Hindell *et al.* 2002; Hindell 2006). Atherinids and gobies have previously been recognised as important fishes in the diet, while amphipods, mysid shrimp and larger epibenthic shrimp (e.g. *Macrobrachium*) were the main crustaceans consumed. In the current study, the pelagic clupeid, sandy sprat, was the most important prey item for Australian salmon. This prey species made up a considerable proportion by weight of juvenile eastern Australian salmon diet (<200 mm FL) in northern NSW, southern NSW and Victoria (30.3%, 19.3% and 18.3%, respectively) (Stewart *et al.* 2011). Sandy sprat was also a common diet item in larger (190–340 mm SL) western Australian salmon in Western Port, Victoria (Hoedt and Dimmlich 1994).

A pronounced shift in diet was observed for western Australian salmon; diet changed from a mix of teleosts and crustaceans (amphipods and mysid shrimp) at ~85 mm to a diet comprised of almost only teleosts by ~190 mm, supporting our hypothesis that Australian salmon would exhibit an ontogenetic shift from small crustaceans to fish. While the literature strongly supports an ontogenetic shift to a piscivorous diet for the species (Cappel 1987; Hoedt and Dimmlich 1994; Hindell 2006; Stewart *et al.* 2011), a strong seasonal decline in the abundance of crustaceans such as amphipods from December to March in the Coorong (Dittmann *et al.* 2014b) may have also influenced observed diet composition trends.

Limitations and further research

Australian salmon do not spawn in SA waters, instead western Australian salmon and eastern Australian salmon spawn in southwest WA and southern NSW, respectively, and currents transport eggs and larvae to SA (Malcolm 1960; Nicholls 1972; Stanley and Malcolm 1977; Cappel 1987). Due to differences in the time of spawning between species, juvenile western Australian salmon settle in SA approximately 4–6 months before eastern Australian salmon (Jones 1999). Consequently, it was not appropriate to assess differences between species as

similar-sized juveniles of the two species occurred during different months, when benthic invertebrate prey abundances differed (Dittmann *et al.* 2014b). Similarly, sampling for this project did not occur past March; therefore, only small juveniles (<100 mm TL) from the eastern species were caught. To improve our understanding of the relative importance of both species in the Coorong and their diets, more comprehensive studies are required. Such studies should have a duration that extends beyond March to incorporate larger eastern Australian salmon (>100 mm), and interpret diets in relation to the ambient prey assemblage given that the prey assemblages may show seasonal fluctuations (Geddes 2005; Dittmann *et al.* 2006; Rolston and Dittmann 2009).

PART 2 STABLE ISOTOPE ANALYSIS OF COORONG FOOD-WEB

2.1 Background

To strengthen and support findings from the stomach-content analysis (Part 1), a stable isotope project (focussing on congolli, *Pseudaphritis urvillii*) was carried out by Johnson (2014), also allowing for an investigation into trophic structure of the Coorong. While this study was resource-limited compared to the 2007 Coorong food-web study (Deegan *et al.* 2010), it provides a good basis for understanding the food-web and trophic links under current (2013-14) moderate inflow conditions. A review of the key findings from Johnson (2014) and further interpretation of the Coorong food-web (e.g. trophic guilds) based on the data from the stable isotope study is provided in the following sections.

2.2 Methods

Various fish (consumer) and macroinvertebrates (potential prey) were collected during March 2014 from 10 sites within two regions of the Coorong (Murray Estuary and North Lagoon) spanning from Monument Road (Beacon 19) to Noonameena (refer to Johnson 2014). This sampling period coincided with fish sampling for stomach-content analysis (Part 1). Muscle tissue from fish and whole invertebrates were freeze-dried, ground and then analysed for carbon ($\delta^{13}\text{C} = {}^{13}\text{C}:{}^{12}\text{C}$) and nitrogen ($\delta^{15}\text{N} = {}^{15}\text{N}:{}^{14}\text{N}$) stable isotope ratios using a mass spectrometer (Johnson 2014). Refer to Johnson (2014) for detailed methods for stable isotope sampling, preparation and analyses.

$\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ bi-plots (regions separately) containing fish and potential prey items were constructed to assess the trophic structure of the Coorong and to estimate the diets of key fish species. As previously described, the carbon isotopic signature of a consumer ($\delta^{13}\text{C}$) does not differ much (<1‰) from its food, but nitrogen ($\delta^{15}\text{N}$) is typically more enriched in the consumer (~3.4‰) (DeNiro and Epstein 1978; Peterson and Fry 1987). Food items with: 1) $\delta^{15}\text{N}$ signatures that were ~3.4‰ (i.e. one trophic position) more depleted than the consumer; and 2) $\delta^{13}\text{C}$ signatures that were within 1‰ of the consumer were considered to be potential prey items of that consumer and are summarised in Table 10 for each species. It is important to note that $\delta^{13}\text{C}$ of a consumer is an integration of all prey and thus, may not directly align with particular prey items.

To give an indication of the feeding mode of fish species and other taxonomic groups, trophic positions (TP) were calculated for the subject using $\delta^{15}\text{N}$ in the following equation. Given that no primary consumers were collected in this project, the average $\delta^{15}\text{N}$ of known secondary consumers, i.e. trophic level 2 (e.g. the filter-feeding tubeworm *Ficopomatus enigmaticus*, deposit-feeding polychaetes, *Australonereis ehlersi* and Family Capitellidae etc.) was used as a baseline reference to calculate the trophic position of the subject. The standard trophic enrichment mean of 3.4‰ (DeNiro and Epstein 1978) was used to represent the enrichment ($\delta^{15}\text{N} = {}^{15}\text{N}:{}^{14}\text{N}$) from one trophic level to another.

$$TP_{subject} = \frac{\delta^{15}N_{subject} - \delta^{15}N_{baseline}}{3.4} + 2$$

2.3 Results

2.3.1 Food-web structure and trophic guilds

Stable nitrogen ($\delta^{15}\text{N}$) and carbon ($\delta^{13}\text{C}$) isotope bi-plots for the Murray Estuary and North Lagoon regions are presented in Figure 7 for those taxa that were collected in sufficient quantities. Large-bodied fish species have been separated into different size classes to account for potential ontogenetic shifts in diet (Elliott *et al.* 2002; Part 1). Many taxa had similar $\delta^{15}\text{N}$ signatures, which is indicative of them occupying the same ‘trophic guilds’. These trophic guilds have been described for each of the two regions below.

Murray Estuary

Most benthic invertebrates of the Murray Estuary fell into the ‘primary consumer’ group (approximately trophic level 2). These invertebrates included the nereid polychaete, *Australonereis ehlersi*, the bivalve, *Soletellina* sp., the tubeworm, *Ficopomatus enigmaticus*, and amphipods (Figure 7). Variation in $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ was high for amphipods, which encompassed multiple families. The gastropod, *Salinator fragilis*, had a slightly more enriched $\delta^{15}\text{N}$ value compared to other primary consumers. The wide range in $\delta^{13}\text{C}$ isotopic signatures suggests that these primary consumers are deriving their organic matter from a variety of sources.

‘Predatory and omnivorous invertebrates’ (i.e. invertebrates with some form of carnivory), formed a group between trophic level 2.5 and 3. These included the polychaetes, *Phyllodoce* sp., *Simplisetia aequisetis* and *Aglaophamus australiensis*, and the crab, *Paragrapsus gaimardii*. The high trophic level of *Salinator fragilis* and overlap of amphipod $\delta^{15}\text{N}$ into the

omnivorous category suggests that *Salinator fragilis* and amphipods (or certain species) may also be feeding omnivorously. Compared to other invertebrates, the trophic position of *Paragrapsus gaimardii* indicated the greatest level of carnivory.

Trophic positions of large yellow-eye mullet (*Aldrichetta forsteri*) (>200 mm), sandy sprat (*Hyperlophus vittatus*) and bony herring (*Nematalosa erebi*) showed some level of omnivory, suggesting these species are 'omnivorous teleosts'. Bony herring indicated greater omnivory (mean trophic position = ~2.5), compared to the other two species, which are closer to the 'secondary consumer' group (trophic level 3). Small congolli (<80 mm) also fell within this group.

The majority of teleosts were situated between trophic level 3 and 4, indicating carnivorous feeding ('carnivorous teleosts'). Many species (e.g. <100 mm Australian salmon (*Arripis trutta*), <400 mm mulloway (*Argyrosomus japonicus*) and >200 mm greenback flounder (*Rhombosolea tapirina*)) exhibited variability in $\delta^{15}\text{N}$ signatures, suggesting they fed on a variety of animal groups including primary consumers, predatory and omnivorous invertebrates, and other teleosts. Gobies (Gobiidae) had the lowest mean trophic position of this group, which indicated predominant feeding on primary consumers.

Large mulloway (>700 mm) was the group with the highest trophic position and the only group that had a trophic position above level 4 ('higher-ordered carnivore'). This may suggest a high proportion of teleosts and/or predatory polychaetes in diet composition. There was almost no variation in $\delta^{15}\text{N}$ between the two large mulloway samples.

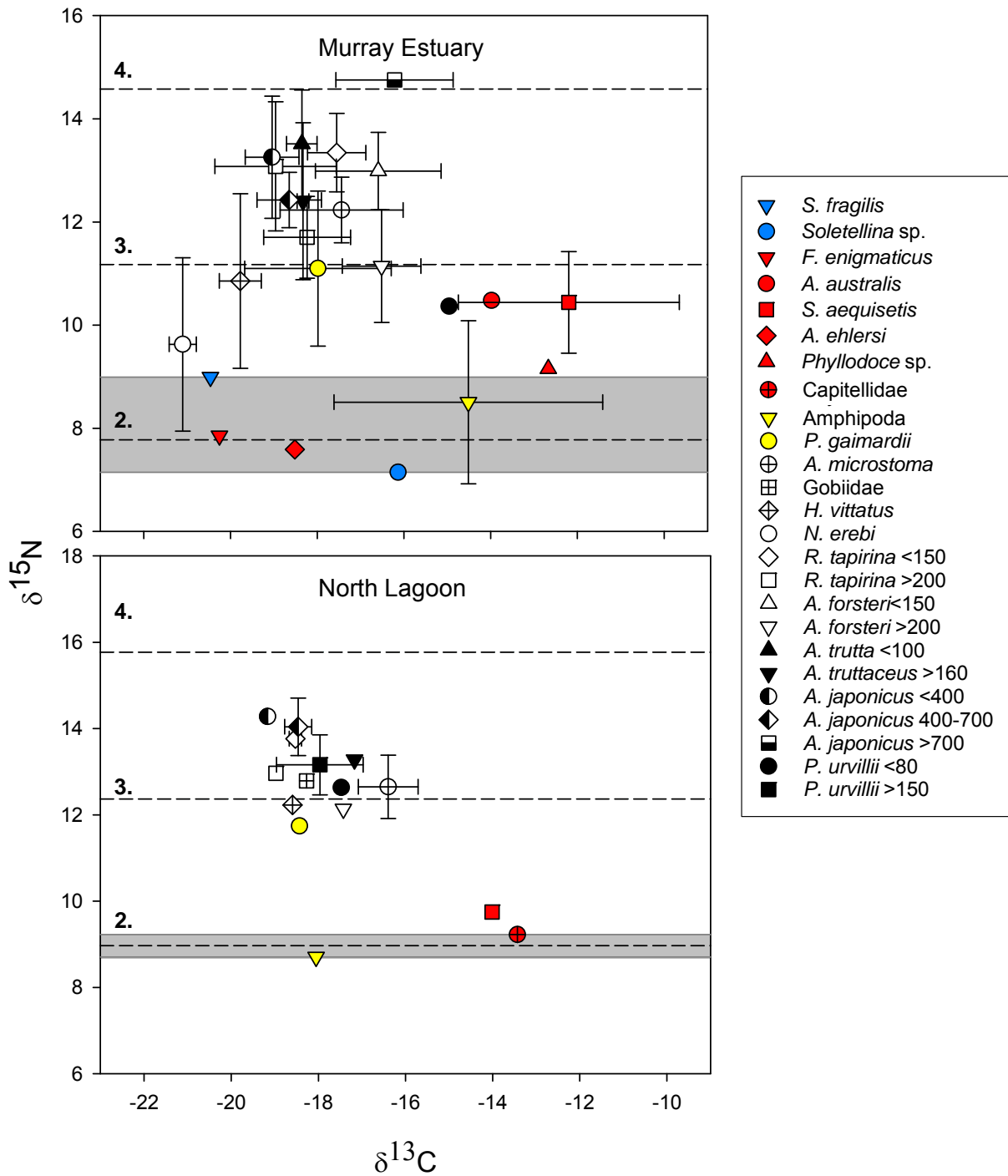


Figure 7. Stable nitrogen ($\delta^{15}\text{N}$) and carbon ($\delta^{13}\text{C}$) isotope signatures of various teleosts (white or black), molluscs (blue), polychaetes (red) and crustaceans (yellow) in the Murray Estuary and North Lagoon of the Coorong (adapted from Johnson (2014)). Trophic levels (boundaries indicated by dotted lines) are presented for $\delta^{15}\text{N}$ in increments of $\sim 3.4\text{‰}$ with the highlighted grey box representing the range of primary consumers in trophic level two. Teleost size classes are in total length (mm). Error bars represent the standard deviations.

North Lagoon

Interpretation of results for the North Lagoon should be treated with caution due to missing target prey and consumer species and low sample numbers. In some cases, single samples allowed no estimation of error in $\delta^{15}\text{N}$ or $\delta^{13}\text{C}$ signature for certain species.

Benthic invertebrates of the North Lagoon fell into the 'primary consumer' group (around trophic level 2). These invertebrates included the nereid polychaete, *Simplisetia aequisetis*, capitellid polychaetes and amphipods (Figure 7). The only member of the 'omnivorous invertebrate' group formed between trophic level 2.5 and 3 was the crab, *Paragrapsus gaimardii*. Large yellow-eye mullet (>200 mm) represented the 'omnivorous teleost' group. Sandy sprat, smallmouthed hardyhead (*Atherinosoma microstoma*), congolli, greenback flounder, small yellow-eye mullet (<150 mm) and mulloway fell between trophic level 3 and 3.5, indicating 'carnivorous feeding' for these groups. Trophic positions suggest primary consumers are the main food sources for these fish species. Gobies had low variation in $\delta^{15}\text{N}$ signatures. Small (<400 mm) and medium (400–700 mm) mulloway had the highest trophic positions.

2.3.2 Trophic position and fish diet estimation

Trophic positions of taxa and potential diet items for fish species are provided in Table 10. It is important to note that some macroinvertebrate prey items were not collected in sufficient quantities for analysis and primary producers (i.e. phytoplankton, macroalgae and macrophytes) and zooplankton were not sampled. These items have not been included in the interpretation of fish diet.

The tubeworm, *Ficopomatus enigmaticus*, was identified as a potential dietary item for sandy sprat and bony herring in the Murray Estuary. Being a filter-feeder, this prey item would have a signature close to zooplankton, which were not sampled in the project. Amphipods were identified as a key food source for many small carnivorous teleosts (e.g. gobies, smallmouthed hardyhead and congolli), while *Paragrapsus gaimardii* was identified as a key food source for most large-bodied species (e.g. Australian salmon, greenback flounder and mulloway). In the Murray Estuary, sandy sprat were likely to be involved in the diet of large greenback flounder, mulloway (<700 mm) and Australian salmon. Large yellow-eye mullet were also identified as a potential food source for medium (400–700 mm) mulloway of the North Lagoon and large mulloway (>700 mm) of the Murray Estuary. For some groups within the large-bodied species

there were no potential prey items that could be identified, as most prey items collected were outside the boundaries of the specified prey range (Table 10; Figure 7).

Table 10. Mean trophic positions (\pm S.D. where applicable) for all taxa and prey items that were identified by stable isotope analysis to be involved in the diet of fish. Regions are ME = Murray Estuary, NL = North Lagoon.

Species	Size class (mm)	Region	Mean trophic position	Trophic group	Potential prey items
<i>Australonereis ehlersi</i>	N/A	ME	1.91	Primary consumer	
<i>Soletellina</i>	N/A	ME	1.78	Primary consumer	
<i>Ficopomatus enigmaticus</i>	N/A	ME	1.99	Primary consumer	
Amphipoda	N/A	ME	2.18 \pm 0.46	Primary consumer	
	N/A	NL	1.91	Primary consumer	
<i>Salinator fragilis</i>	N/A	ME	2.32	Primary consumer	
Capitellidae	N/A	NL	2.07	Primary consumer	
<i>Phyllodoce</i> sp.	N/A	ME	2.37	Predatory and omnivorous invertebrate	
<i>Simplisetia aequisetis</i>	N/A	ME	2.74 \pm 0.29	Predatory and omnivorous invertebrate	
	N/A	NL	2.22	Primary consumer	
<i>Aglaophamus australiensis</i>	N/A	ME	2.76	Predatory and omnivorous invertebrate	
<i>Paragrapsus gaimardii</i>	N/A	ME	2.94 \pm 0.44	Predatory and omnivorous invertebrate	
	N/A	NL	2.81	Predatory and omnivorous invertebrate	
Bony herring	N/A	ME	2.50 \pm 0.49	Omnivorous teleost	<i>Ficopomatus enigmaticus</i>
Sandy sprat	N/A	ME	2.87 \pm 0.54	Omnivorous teleost	<i>Ficopomatus enigmaticus</i> , <i>Salinator</i> sp.
	N/A	NL	2.95	Carnivorous teleost	Amphipods
Yelloweye mullet	<150	ME	3.50 \pm 0.22	Carnivorous teleost	Amphipods, <i>Paragrapsus gaimardii</i> , small congolli (<100 mm), large yellow-eye mullet (>200 mm)
	<150	NL	3.26 \pm 0.06	Carnivorous teleost	None identified
	>200	ME	2.95 \pm 0.32	Omnivorous teleost	Amphipods, <i>Soletellina</i> sp., <i>Australonereis ehlersi</i>
	>200	NL	2.92 \pm 0.25	Omnivorous teleost	Amphipods
Gobies	N/A	ME	3.12 \pm 0.23	Carnivorous teleost	Amphipods, <i>Australonereis ehlersi</i>
	N/A	NL	3.11 \pm 0.02	Carnivorous teleost	Amphipods
Smallmouthed hardyhead	N/A	ME	3.27 \pm 0.19	Carnivorous teleost	Amphipods
	N/A	NL	3.07 \pm 0.22	Carnivorous teleost	Amphipods
Congolli	<80	ME	2.72	Omnivorous teleost	Amphipods, <i>Soletellina</i> sp.
	<80	NL	3.07 \pm 0.08	Carnivorous teleost	Amphipods
	>150	NL	3.22 \pm 0.20	Carnivorous teleost	Amphipods

Species	Size class (mm)	Region	Mean trophic position	Trophic group	Potential prey items
Greenback flounder	<150	ME	3.60 ± 0.22	Carnivorous teleost	<i>Paragrapsus gaimardii</i> , amphipods, large yellow-eye mullet (>200 mm)
	<150	NL	3.40 ± 0.00	Carnivorous teleost	None identified
	>200	ME	3.52 ± 0.37	Carnivorous teleost	Sandy sprat, <i>Paragrapsus gaimardii</i> , amphipods, <i>Salinator</i> sp.
	>200	NL	3.17	Carnivorous teleost	None identified
Australian salmon	<100 <i>A. trutta</i>	ME	3.32 ± 0.45	Carnivorous teleost	<i>Paragrapsus gaimardii</i> , amphipods, sandy sprat, large yellow-eye mullet (>200 mm)
	>160 <i>A. truttaceus</i>	ME	3.65 ± 0.31	Carnivorous teleost	<i>Paragrapsus gaimardii</i> , gobies, sandy sprat, large yellow-eye mullet (>200 mm), amphipods
Mulloway	<400	ME	3.58 ± 0.35	Carnivorous teleost	Sandy sprat, <i>Paragrapsus gaimardii</i> , gobies, small Australian salmon (<100 mm), <i>Salinator</i> sp.
	<400	NL	3.55	Carnivorous teleost	None identified
	400–700	ME	3.33 ± 0.16	Carnivorous teleost	Sandy sprat, amphipods, <i>Paragrapsus gaimardii</i>
	400–700	NL	3.48 ± 0.20	Carnivorous teleost	Large yellow-eye mullet (>200 mm)
	>700	ME	4.01 ± 0.00	Higher-ordered carnivore	Large yellow-eye mullet (>200 mm), <i>Paragrapsus gaimardii</i> , gobies, smallmouthed hardyhead

2.4 Discussion

2.4.1 Food-web structure and trophic guilds

In the current stable isotope analysis of the Coorong food-web (Johnson 2014), trophic groups of taxa were identified based on trophic positions: primary consumers (trophic level 2), omnivorous and predatory invertebrates (trophic level 2.5–3), omnivorous teleosts (trophic level 2.5–3), carnivorous teleosts (trophic level 3–4) and higher-ordered carnivores (above trophic level 4). Members that were allocated to those groups align well with their known feeding modes (e.g. Fauchald and Jumars 1979; Elliott *et al.* 2007; Jumars *et al.* 2015) and support the use of stable isotope analyses as a powerful tool in exploring the trophic structure of estuarine food-webs.

2.4.2 Fish diet estimation

As many species of fish in the Coorong are known to feed at various trophic levels (Deegan *et al.* 2010; Giatas 2012; Earl 2014), the isotopic signatures of consumers may not necessarily correspond to their primary food sources and/or may misleadingly predict unimportant food sources. For example, large yellow-eye mullet (>200 mm) are omnivorous and occupied a trophic position between 2.5 and 3, similar to that of predatory/omnivorous invertebrates. Due to the low trophic position of large yellow-eye mullet, the isotope evidence alone misclassifies them as a potential food source for juvenile conspecifics (<150 mm), as well as juvenile greenback flounder (<150 mm), which is implausible. Additionally, there is the complexity associated with the possibility that some individuals may have previously fed in different regions, or a completely different environment (e.g. marine environment) prior to capture and thus, may not reflect isotopic signatures of prey from the region of capture. All these factors must be considered when interpreting stable isotope results. Nevertheless, stable isotope analysis provided complementary results for the stomach-content analysis of the three target species (Part 1), which will be compared below.

Mulloway

Diet estimation from stable isotope analysis identified sandy sprat as the principal food source for small- and medium-sized mulloway (<700 mm) from the Murray Estuary. Large yellow-eye mullet (>200 mm) were identified as the most likely food source for large mulloway (>700 mm) from the Murray Estuary. These results are consistent with stomach-content findings in Section 1.1. In the absence of large yellow-eye mullet (>200 mm), stable isotope analysis suggests that crabs (*Paragrapsus gaimardii*), gobies and smallmouthed hardyhead could be the main prey sources for large mulloway (>700 mm). These items were common items in the medium size class (400-700 mm) and potentially contributed to diet of large mulloway.

For the North Lagoon, few prey items were identified for mulloway as they occurred outside the bounds of the predicted prey range. Missing and/or low sample numbers of target prey species and the high mobility of mulloway likely prevented diet estimation for this species in the North Lagoon. The observation that *Paragrapsus gaimardii* was the key item in the stomachs of medium-sized mulloway of the North Lagoon (Section 1.1), and that stable isotope results suggest it to be a potential item in medium-sized mulloway diet in the Murray Estuary may also be explained by fish having fed in a different region to that in which they were caught.

Small- and medium-sized mulloway occupied a similar trophic position of ~3.5, which classified them as a carnivorous species. Large mulloway occupied a higher trophic position (4.01) suggesting that they were feeding on prey higher in the food chain ('higher-ordered carnivore'). These classifications are consistent with the literature on the species (Whitfield and Blaber 1978; Marais 1984; Griffiths 1997; Taylor *et al.* 2006a) and findings from the stomach-content study (Section 1.1).

The stable isotope analysis indicated a trophic ($\delta^{15}\text{N}$) and carbon pathway ($\delta^{13}\text{C}$) shift for mulloway from <700 mm to >700 mm for the Murray Estuary region. This carbon pathway shift was consistent with stomach-content results (Section 1.1) where diet shifted from $\delta^{13}\text{C}$ -depleted omnivorous/zooplanktivorous teleosts (sandy sprat and bony herring; phytoplankton-based food-web) to the more $\delta^{13}\text{C}$ -enriched large-bodied, omnivorous teleost, yellow-eye mullet (benthic/detritus-based food-web). Isotopic signatures of $\delta^{15}\text{N}$ became more enriched, suggesting an increase in trophic position, which was consistent with stomach-content results (Section 1.1). The key prey items of medium mulloway (i.e. bony herring and sandy sprat) had lower $\delta^{13}\text{N}$ values than the key prey item of large mulloway (i.e. yellow-eye mullet).

No major trophic or carbon pathway shift was observed from the <400 to 400–700 mm size class; however, the mean trophic position (3.33, 3.48) of medium-sized (400–700 mm) mulloway was slightly less than that (3.58, 3.55) of smaller mulloway (<400 mm) for both regions. Stomach-content results showed a shift in diet from small-bodied teleosts (e.g. sandy sprat) to larger teleosts (e.g. bony herring) for the Murray Estuary and from small crustaceans (e.g. mysid shrimp and amphipods) to large crabs (i.e. *Paragrapsus gaimardii*) in the North Lagoon. As the major prey items of these two different size classes had similar isotopic signatures it would be expected that the consumers would also have similar isotopic signatures, which is reflected in the stable isotope bi-plots.

Congolli

Amphipods were identified by stable isotope analysis as the main food source for congolli for all sizes and regions. This provides support for stomach-content analysis results, where amphipods were the most common and abundant prey in the diet of congolli (Section 1.2). Stomach-content results also show that polychaetes (i.e. capitellids and nereids), mysid shrimp and teleosts are important in the diet of congolli. The wider variation in $\delta^{13}\text{C}$ for large congolli of the North Lagoon suggests a wider variation in diet of fish within the region, which was supported by stomach-content analysis (Section 1.2).

Small congolli from the Murray Estuary occupied a low trophic position (2.72), which classified them as an omnivorous species, while those from the North Lagoon occupied a trophic position of ~3–3.5, which classified them as a carnivorous species. Carnivorous feeding is consistent with the literature on the species (Hortle and White 1980) and findings from the stomach-content study (Section 1.2). There is no evidence to suggest that congolli are omnivorous as juveniles (Hortle and White 1980), therefore the reason for the low trophic position observed in this study remains unclear. In the North Lagoon, both sizes of congolli had similar trophic positions, indicating that they were feeding at the same trophic level. These results are consistent with stomach-content observations (amphipods and herbivorous/omnivorous polychaetes) (Section 1.2).

Australian salmon

Stomach-content results generally supported stable isotope diet estimation for Australian salmon (i.e. amphipods, sandy sprat, gobies) (Section 1.3), with the exception of *Paragrapsus gaimardii* and large yellow-eye mullet (>200 mm). While it is possible that juvenile *Paragrapsus gaimardii* may be a food source for Australian salmon, it is unlikely that large yellow-eye mullet (>200 mm) were. Stomach-content results suggest smallmouthed hardyhead and copepods were other important items prey items for the species.

Australian salmon occupied a trophic position between 3.3 and 3.7, indicating that they are a carnivorous species. This is consistent with the literature (Robertson 1982; Cappo 1987; Hoedt and Dimmlich 1994; Stewart *et al.* 2011) and stomach-content results (Section 1.3). The slight increase in trophic position from <100 mm to >160 mm supports stomach-content observations where the proportion of teleosts increased in the diet, while small crustaceans declined with increasing Australian salmon size (Section 1.3). Both sizes showed a wide variation in $\delta^{15}\text{N}$, suggesting that salmon were feeding at a variety of trophic levels; also supported by the stomach-content results (i.e. mix of carnivorous teleosts and primary consumers) (Section 1.3).

Enriched $\delta^{13}\text{C}$ of small congolli and large mulloway from the Murray Estuary

Large mulloway (>700 mm) and small congolli (<80 mm) had the most $\delta^{13}\text{C}$ -enriched signatures of all teleosts in the Murray Estuary. Although the positions of these groups in the stable isotope bi-plot corresponded to their primary prey item as identified by stomach-content analysis (large yellow-eye mullet and amphipods for mulloway and congolli, respectively), their $\delta^{13}\text{C}$ -enriched signature could also imply food sources were of marine origin (Fry and Sherr 1984). This would

align with migrations/movements from marine to estuarine habitats exhibited by both species. For example, larger (adult) mulloway are mainly found along the ocean beaches and bays of the nearshore marine environment (Griffiths 1996; Ferguson and Ward 2011) and may occasionally enter estuarine waters to exploit food resources (Griffiths 1996). Alternatively, congolli spawn in the marine environment and young-of-the-year (i.e. <60 mm) migrate to the Murray Estuary and upstream into the freshwater environment during spring and summer (Bice *et al.* 2012; Bice and Zampatti 2014).

Stable isotope signatures may therefore reflect prey assimilated from a previous habitat outside of the site/region of capture (i.e. the marine environment). In this regard, turnover rates of carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) isotopes in muscle tissue of fish (i.e. the time it takes for a switch in diet with a different isotopic composition to be reflected into the consumer's tissue) varies with metabolic factors such as body size and growth (Hesslein *et al.* 1993; MacAvoy *et al.* 2001). Larger, older individuals are more likely to have longer turnover times (months) compared to early life-history stages such as larvae (days to weeks) (Hesslein *et al.* 1993). Given that detailed prior movement histories of individuals and turnover rates of isotopes in tissues for the two species are unknown, it is difficult to confirm whether the signatures of small congolli and large mulloway are reflective of prey within the Coorong or reflective of prey from the marine environment.

2.4.3 Limitations and further research

A number of factors such as missing biota, movement of fish and spatial/temporal coverage may have limited diet estimation and overall interpretation of the trophic structure of the Coorong. Limitations of the study are described below.

Diet estimation

Missing potential prey items and low sample sizes of prey items or consumers likely reduced the accuracy of fish diet estimation. For example, the tubeworm, *Ficopomatus enigmaticus*, was identified as the principal item in the diet of sandy sprat and bony bream; however, it is highly unlikely either species would feed on such a prey item as both sandy sprat and juvenile bony herring (Atkins 1984; Medeiros and Arthington 2008) are zooplanktivorous feeders (Bice *et al.* 2015) and do not possess the morphological adaptations to feed on the calcareous-tubed polychaete. Since zooplankton were not collected for stable isotope analysis, we could not estimate the contribution of this prey item to the diet of any fish species, but it is likely that

zooplankton and *Ficopomatus enigmaticus* would occupy similar trophic positions and have similar isotopic signatures as they have similar feeding modes (i.e. primarily suspension feeding on phytoplankton and organic matter) (Fauchald and Jumars 1979).

Due to the high mobility of mulloway (Hall 1986; Silberschneider and Gray 2008), it is possible that individuals may have foraged outside of their capture region and isotopic signatures of tissue may reflect prey from a different region or combination of regions. It is also possible that larger mulloway (>700 mm) and small congolli (<80 mm) may have recently entered the Coorong system after living/feeding outside of the Coorong in the marine environment. As identified in Part 1 (e.g. congolli), a better understanding of fish movements, with regards their foraging ranges and 'site fidelity' of individuals from all three species is required.

Trophic structure of Coorong

No black bream were captured during the March sampling event for stable isotope analysis. This large-bodied species plays a role as a higher-order predator in the Coorong food-web. Other biota (e.g. river garfish, mysid shrimp, zooplankton, phytoplankton, algae etc.) that were not collected during sampling may play important roles in the Coorong food-web and the absence of these in the analysis has likely influenced interpretations of food-web structure and diet estimation.

The primary focus of the stable isotope component was to provide complementary data for the stomach-content analysis of the three target species, therefore this food-web study did not cover the same spatial extent as that seen in Deegan *et al.* (2010) during the drought. Food-web structure is likely to be different further into the salinity gradient of the Coorong (i.e. loss of fish predators) (Deegan *et al.* 2010). Similarly, this study focussed on the spring/summer period and food-web structure may be different during winter. Future isotope studies assessing trophic structure during moderate or high inflows should aim to address these aforementioned limitations.

PART 3 GENERAL DISCUSSION

3.1 Summary of findings

A combination of stomach-content and stable isotope analyses were adopted in the current study to improve our knowledge of the diet and trophic interactions of key fish species in the Coorong, namely mulloway, congolli and Australian salmon, during a moderate inflow year that followed consecutive years of high freshwater inflows. Mulloway demonstrated a pronounced ontogenetic shift in diet from small crustaceans (i.e. mysid shrimp and amphipods) and small-bodied teleost fish (e.g. sandy sprat) (at <400 mm), to crabs and medium-sized teleosts (e.g. congolli and bony herring) (at 400–700 mm), and finally to yellow-eye mullet (at >700 mm). Whilst not investigated in this study, the shift may be due to increased gape size and/or increased mobility with increasing size. Congolli fed mainly on infaunal and epi-benthic crustaceans (i.e. amphipods and polychaetes), while juvenile Australian salmon were mainly piscivorous, feeding on a variety of small-bodied teleosts (e.g. sandy sprat, smallmouthed hardyhead and gobies). Diet varied at a spatial scale for mulloway (regions) and congolli (sites), owing to differences in relative abundances of prey whose distribution may be driven by salinity preferences or other environmental factors (e.g. habitat characteristics). Diet estimation from stable isotope analysis generally provided good support for key diet items found in the stomachs of the three target species. Trophic position estimation was also consistent with known feeding modes of each species.

3.2 Comparison to drought

3.2.1 Mulloway

During the drought, mulloway diet was primarily composed of mullet, hardyhead and crabs at Pelican Point in 2005 (480–660 mm, $n = 20$) (Geddes and Francis 2008), and at Mundoo, Goolwa Channel and Pelican Point in 2007 (unspecified lengths, $n = 4–24$) (Deegan *et al.* 2010). Congolli and gobies were other abundant prey items during 2005 (Geddes and Francis 2008) and 2007 (Deegan *et al.* 2010), respectively. Isosource modelling of stable isotope results in 2007 identified sandy sprat and mullet as potential principal food sources of mulloway (400–600 mm) at Goolwa, whereas crabs, the large polychaete, *Aglaophamus australiensis*, and gobies were deemed more important for mulloway from Pelican Point (Deegan *et al.* 2010).

Mullet, hardyhead, congolli, gobies and crabs were also relatively frequent items for medium-sized mulloway (400–700 mm) during the current study. The broad salinity tolerances of yellow-eye mullet (0–74.1 ppt), smallmouthed hardyhead (0–133.5 ppt), congolli (0–74.1 ppt), Tamar goby (0–60.1 ppt) (Noell *et al.* 2009; Ye *et al.* 2011) and the crab, *Paragrapsus gaimardii* (0–80 ppt) (Brookes *et al.* 2009) suggests these species are well adapted to both moderate inflows and drought conditions. Stable isotope analysis by Deegan *et al.* (2010) identified that sandy sprat may have been a prey item involved in the diet of mulloway at Goolwa, although none were found in their stomachs. Findings from the current study show that sandy sprat is a particularly important item under moderate inflows for small mulloway (<400 mm), and to a lesser degree, medium-sized (400–700 mm) mulloway. The abundance of this prey species appears associated with freshwater inflows, with elevated abundance in the Coorong after recent high flow years (Livore *et al.* 2013). This association may be related to enhanced habitat connectivity, estuarine productivity and trophic subsidy (Bice *et al.* 2015).

Bony herring was an important prey item in the diet of medium-sized mulloway (400–700 mm) during 2013-14 (moderate inflow). This freshwater species was absent in mulloway stomachs (400–600 mm) during the drought period in 2005 and 2007 (Geddes and Francis 2008; Deegan *et al.* 2010) when freshwater inflow through barrages was minimal. The high proportion of bony herring in the diet of mulloway during the current study is consistent with findings by Hall (1986), who assessed diet of mulloway <460 mm in the North Lagoon of the Coorong during high inflows in 1983-84 and found hardyhead (Atherinidae) and bony herring (*Nematalosa erebi*) to be the most common prey items. Bony herring are more abundant in the Coorong during higher freshwater inflows (Livore *et al.* 2013); presumably due to lower salinities and/or transportation of individuals via flow through barrages. These abundances appear to be reflected in the diet composition of mulloway. Mysid shrimp and amphipods were not present in mulloway stomachs during the drought, but were important diet items for small mulloway (<400 mm) in the current study. This is likely a result of a narrow size-range of medium-sized fish sampled during the drought (480–660 mm).

Mulloway were estimated to occupy a high trophic position of ~4–4.5 during the drought (Deegan *et al.* 2010), which was ~1 trophic position higher than the current study (Johnson 2014). This may be a result of a high proportion of omnivorous bony herring (low $\delta^{15}\text{N}$) in the diet of mulloway in the current study. Alternatively, trophic position differences may be due to the differences in trophic positions of large yellow-eye mullet between periods, i.e. large yellow-eye mullet occupied a lower trophic position during the current study (drought, 3.03–3.48; 2013-

14, 2.92–2.95). During the drought at Goolwa, the trophic position of mulloway (400–600 mm) was slightly lower than that of small mulloway (120–190 mm), which was most likely a result of crabs in their diet, noting crabs occupied a lower trophic position (Deegan *et al.* 2010). The same trend was evident in the current study.

3.2.2 Congolli

Congolli (100–170 mm) diet was estimated based on stable isotope data at Long Point in the North Lagoon of the Coorong during the drought in 2007 (Deegan *et al.* 2010). Crabs and polychaetes (genera *Capitella* and *Simplisetia*) were identified as potential key prey items in diet composition. Diet was considered unlikely to have include the nereid polychaete, *Australonereis ehlersi*, juvenile crabs or the tubeworm, *Ficopomatus enigmaticus*. Results from the current stomach-content study (moderate inflow) support those predictions by Deegan *et al.* (2010), with the exception of the nereid, *Australonereis ehlersi*, which made up a reasonable contribution to large congolli diet by volume in the current study. It is unsuitable to draw too many other conclusions from this comparison, given that different methods have been used and only one site (Long Point) has been described for congolli in 2007 (Deegan *et al.* 2010). However, 2013-14 results suggest that amphipods play an important role in the diet of this species during moderate inflow. Disregarding small congolli (<80 mm) from the Murray Estuary in the current study, congolli had similar trophic positions during both flow periods (Deegan *et al.* 2010; Johnson 2014), indicating that they were feeding at the same trophic level (presumably on 'primary consumers').

3.2.3 Trophic structure

A number of factors limited the detail at which the Coorong trophic structure could be compared between the two different flow periods: spatial analysis (i.e. site scale vs. regional scale), salinities, level of sampling, etc. Furthermore, presence/absence and abundance of consumers and prey are not reflected in stable isotope analysis biplots for either flow period; therefore, it is difficult to know the relative importance of biota in the food-web during different flow periods. Nevertheless, there were some differences identified between the two flow periods. These include changes in feeding modes for some biota (e.g. large yellow-eye mullet from carnivorous to omnivorous), a decrease in trophic level of some carnivorous species (e.g. mulloway) and a shift in carbon ($\delta^{13}\text{C}$) signature of most teleosts.

There was a general shift in $\delta^{13}\text{C}$ from a $\delta^{13}\text{C}$ -enriched ($\sim -16\text{‰}$ to -10‰) to $\delta^{13}\text{C}$ -depleted ($\sim -20\text{‰}$ to -16‰) signature for teleost fishes from drought to 2013-14, which followed consecutive years of high freshwater inflows. Terrestrial food sources are typically $\delta^{13}\text{C}$ -depleted compared to those in freshwater, which are $\delta^{13}\text{C}$ -depleted compared to marine sources (Fry and Sherr 1984). These observations could suggest that there was a greater influence of freshwater-derived organic carbon during 2013-14, which has been transferred up the food chain to higher predators (carnivorous fish). Alternatively, the shift in carbon signature could be an indication of a shift in the proportion of carbon source from benthic production (benthic micro- and macroalgae, and detritus-based) to pelagic production (phytoplankton-based) (France 1995). The greater importance of zooplanktivorous fish such as sandy sprat and juvenile bony herring in predatory fish diet (i.e. mulloway and Australian salmon) during current moderate flow conditions would support this hypothesis.

3.3 Conclusions and management implications

Analysis of the stomach-contents of three carnivorous fish species (mulloway, congolli and Australian salmon) and an accompanying stable isotope analysis of the food-web has improved our understanding and knowledge of food-web structure of the Coorong during a moderate freshwater inflow year that followed consecutive years of high freshwater inflows. This study, in conjunction with Johnson (2014) has provided the first published dietary information for congolli in SA. Stable isotope analysis proved to be a useful tool for understanding the general feeding modes and overall trophic structure; however, to enable a more accurate estimation of diet, complementary stomach-content analysis was required. In order to make a more detailed assessment of changes in the Coorong food-web structure in context of variation in freshwater inflow, a quantitative assessment would be required considering the abundance of prey and predatory assemblage and their trophic position in the food-web.

Management implications

The Coorong is a dynamic environment and species within this system demonstrate fluctuations in abundance and distribution, primarily as a result of variability in freshwater inflow. Data collected in the current study represents diet of the three target species at a point in time during moderate freshwater inflow ($\sim 1,830$ GL/year; mean $\sim 5,000$ ML/day) to the Coorong. The freshwater inflow experienced during this current study is likely to be similar to the most frequent flow scenario expected in the future (i.e. a basin plan target of a minimum of 2,000 ML/day to the Coorong supported by environmental watering). Changes to freshwater inflow has the

potential to affect productivity and modify trophic interactions of these species, as supported by exploratory comparisons of data from this study to other diet and food-web studies conducted in the Coorong during the drought, and must be considered in context of the food-web.

A diverse and abundant prey assemblage is beneficial to all members of the Coorong, particularly for species that have narrow prey ranges or rely on particular prey for different life stages. Regular freshwater inflows to the Coorong and the maintenance of freshwater–estuarine connectivity are recommended and will promote productivity, through nutrient or zooplankton input, and species diversity (e.g. presence of catadromous and freshwater species). For example, sandy sprat, bony herring and amphipods were important prey items in the diets of the three species and are all directly or indirectly associated with freshwater inflows to the Coorong (Dittmann *et al.* 2013; Livore *et al.* 2013; Bice *et al.* 2015).

From a trophic perspective, maintaining the mulloway population in the Coorong is essential as this species is a top predator and thus, plays an important role in maintaining ecosystem structure and stability. The ontogenetic shift in diet shown by this species highlights the importance of considering a diverse prey assemblage for different stages of the species' life history. The Coorong is an important nursery for mulloway in SA and although large mulloway (>700 mm) may also utilise the Coorong, smaller mulloway (i.e. <550 mm) dominate the assemblage structure (Earl and Ward 2014). Maintaining habitat quality and food resources (e.g. sandy sprat, small crustaceans, crabs, congolli and bony herring) within this nursery ground will subsequently contribute to the resilience of the broader mulloway population.

Congolli is an important catadromous fish species with significant ecological and conservation values in the Coorong and Lower Lakes. Results from the current study suggest that small congolli (<80 mm) may be reliant on amphipods as their primary food source, notwithstanding this prey was abundant during moderate freshwater inflows. Disturbance to the benthic invertebrate community of the Coorong, through annual variations in hydrology, has the potential to disrupt feeding behaviour of congolli.

Australian salmon are marine estuarine-opportunists that utilise the Coorong estuary as a nursery area, exploiting food resources and gaining protection from predators. Australian salmon demonstrated the ability to feed upon the benthos (e.g. gobies) and in the water column (e.g. sandy sprat). Changes in abundance and distribution of prey items in the Coorong, which may be influenced by hydrology, has the potential to alter Australian salmon diet and feeding

habits (e.g. pelagic vs. benthic feeding), which, in turn, can influence trophic pathways of the food-web.

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APPENDICES

Appendix 1. Site-specific catch summary for mullet. Sample numbers are given for: small (S), <400 mm; medium (M), 400–700 mm; and large (L), >700 mm mullet. Sites are Beacon 19 (B19), Murray Mouth to Godfrey's Landing (MM-GL), Boundary Creek Structure (BCS), Ewe Island to Snake Pit (EI-SP), Pelican Point to (and including) Mark Point (PP-MP), Mark Point to Long Point (MP-LP) and Long Point to Rob's Point (LP-RP).

Region	S	M	L	TOTAL
ME				
B19	5			5
MM-GL		4		4
BCS	35	6		41
EI-SP	1	43	26	69
ME Total	41	53	26	120
NL				
PP-MP	53	27	12	92
MP-LP		14		14
LP-RP	2	35	2	39
NL Total	55	76	14	145
TOTAL	96	129	40	265

Appendix 2. Sampling sites and sampling methods used for the Murray Estuary, North Lagoon and Lower Lake regions. Methods are: SS = standard seine net, MPG = multi-panel gill net, C = commercial mulloway gill nets, DF = double-winged fyke nets and SF = single-winged fyke nets. Months are N = November 2013, J = January 2014 and M = March 2014.

Region	Site	Latitude (°S)	Longitude (°E)	Methods	Month
Murray Estuary	Beacon 19	35.534	138.832	SS, MPG	N, J*, M
	Sugars Beach	35.548	138.877	SS	N, J
	Boundary Creek Structure	35.556	138.934	SS, MPG	N, J*, M
	Boundary Creek Mouth	35.564	138.923	SS	N, M
	Godfrey's Landing	35.568	138.932	SS, C	N, J*, M*
	Tauwitcherie	35.567	138.969	DF	N, J
	Ewe Island to Snake Pit	35.558–35.580	138.950–138.989	C	N, J
North Lagoon	Mark Point	35.638	139.076	SS, MPG	N, J, M
	Long Point	35.693	139.166	SS	N, J, M
	Noonameena	35.757	139.232	SS, MPG	N, J*, M
	Pelican Point to Mark Point	35.595–35.638	139.014–139.076	C	N, M
	Mark Point to Long Point	35.638–35.693	139.076–139.166	C	M
	Long Point to Robs Point	35.693–35.787	139.166–139.286	C	J
Lower Lakes	Steamer Drain	35.533	138.910	SF	N
	Holmes Creek	35.538	138.922	SF	N
	Hunters Creek	35.528	138.898	SF	N
	Finnis Junction	35.479	138.887	SF	N

*There were no MPGs used at Beacon 19 and Noonameena during January, no SS used at Boundary Creek Structure in January and no Cs used at Godfrey's Landing in January or March.

Appendix 3. Importance of prey items in the stomachs of mulloway by region x size as determined by the Index of Relative Importance (IRI, expressed as a percentage) and the percentage contribution of prey items by weight (%W). Regions are the Murray Estuary (ME) and the North Lagoon of the Coorong (NL). %IRI is calculated using the IRI values from Gobiidae and Nereididae, not from individual prey items under these categories, hence these items do not have %IRI values presented. Major groups are in bold. %IRI for each major group is calculated as the sum of prey item under respective groups.

Family (or higher)	Prey items	Size class (mm)											
		<400				400–700				>700			
		ME		NL		ME		NL		ME		NL	
	%W	%IRI	%W	%IRI	%W	%IRI	%W	%IRI	%W	%IRI	%W	%IRI	
Teleostei		96.1	97.3	61.5	12.2	96.3	82.1	64.3	41.0	100.0	100.0	97.8	99.1
Arripidae	<i>Arripis truttaceus</i>					1.8	0.2						
Atherinidae	<i>Atherinosoma microstoma</i>	9.1	0.7	13.6	2.8	0.2	0.1	2.8	6.1				
Clupeidae	<i>Hyperlophus vittatus</i>	37.0	81.0	10.8	7.1	1.6	10.4						
	<i>Nematalosa erebi</i>	39.8	4.7	34.4	0.7	23.0	26.8	7.6	3.9			0.1	0.4
	<i>Sardinops sagax</i>					1.9	0.1						
Engraulidae	<i>Engraulis australis</i>							0.2	<0.1				
Gobiidae		0.2	<0.1			7.4	9.5	2.4	0.3				
	<i>Afurcagobius tamarensis</i>					6.0		2.0					
	<i>Arenigobius bifrenatus</i>					0.3		0.4					
	Gobiidae Unid.	0.2				1.1							
Mugilidae	<i>Aldrichetta forsteri</i>					46.2	12.3	16.9	4.6	100.0	100.0	80.6	94.8
Percidae	<i>Perca fluviatilis</i>					2.9	0.3	0.8	0.1				
Pleuronectidae	Pleuronectidae Unid.					0.5	0.1	0.4	0.1				
Pseudaphritidae	<i>Pseudaphritis urvillii</i>			0.2	<0.1	1.6	0.4	27.3	23.7				
Retropinnidae	<i>Retropinna semoni</i>					<0.1	0.3						
Sciaenidae	<i>Argyrosomus japonicus</i>					3.5	0.2	3.1	0.1			13.3	3.3
Teleostei Unid.		9.9	10.9	2.6	1.6	5.6	21.3	2.9	2.2			3.8	0.6
Crustacea (Malacostraca)		2.2	2.0	36.7	87.3	3.7	17.9	34.1	59.0			2.2	0.9
Amphipoda	Amphipoda (all)	0.1	0.3	3.3	41.7	0.1	5.7						
Decapoda													
Brachyura	Crab unid.			1.4	0.2	<0.1	<0.1						
Grapsidae	<i>Paragrapsus gaimardii</i>	0.2	<0.1	17.4	1.7	3.1	1.6	34.1	59.0			1.9	0.5
Hymenosomatidae	<i>Amarinus lacustris</i>	0.1	<0.1	0.8	0.1								
Portunidae	<i>Ovalipes australiensis</i>											0.3	0.4
Caridea (Palaemonidae)	<i>Macrobrachium</i> sp.	1.3	0.1										
Unid. Decapod (prawn)						0.2	<0.1						
Mysidacea	Mysidacea (all)	0.6	1.6	13.7	43.6	0.2	10.5						
Polychaeta		1.7	0.7	0.9	0.4	<0.1	<0.1	<0.1	<0.1				
Nereididae		1.5	0.6	0.9	0.4			<0.1	<0.1				
	<i>Australonereis ehlersi</i>	<0.1											
	<i>Simplisetia aequisetis</i>	0.2		0.1									
	Nereid Unid.	1.3		0.8				<0.1					
Nephtyidae	<i>Aglaophamus australiensis</i>					<0.1	<0.1						
Polychaeta Unid.	Polychaeta Unid.	0.2	0.1										
Insecta	Lepidoptera (moth)					<0.1	<0.1						
Macroalgae*	Macroalgae Unid.			<0.1	N/A			1.6	N/A				
Unidentified matter*				0.8	N/A								
	<i>n</i> =	41		55		53		76		26		14	

Appendix 4. Month-specific catch summary. Sample numbers are given for: small (S), <400 mm; medium (M), 400–700 mm; and large (L), >700 mm mulloway.

Region	S	M	L	TOTAL
ME				
NOV	3	39	3	45
JAN	24	12	23	59
MAR*	14	2		16
ME Total	41	53	26	120
NL				
NOV	24	15	9	48
JAN	28	34	2	64
MAR	3	27	3	33
NL Total	55	76	14	145
TOTAL	96	129	40	265

*Commercial fishermen were not operating within the Murray Estuary region during March 2014, therefore sample numbers are low for medium- and large-sized mulloway.

Appendix 5. Percentage contribution of prey items by frequency (%F) and number (%N) to total frequency and number for three different size classes of mulloway for the Murray Estuary and North Lagoon of the Coorong. Regions are not separated.

Family (or higher)	Prey items	Size class (mm)					
		<400		400–700		>700	
		% F	%N	% F	%N	% F	%N
Teleostei		72.9	24.1	79.8	66.8	97.5	96.2
Arripidae	<i>Arripis truttaceus</i>			1.6	0.3		
Atherinidae	<i>Atherinosoma microstoma</i>	11.5	2.3	6.2	16.3		
Clupeidae	<i>Hyperlophus vittatus</i>	49.0	16.8	7.8	10.6		
	<i>Nematalosa erebi</i>	5.2	0.2	19.4	11.7	2.5	1.9
	<i>Sardinops sagax</i>			0.8	0.3		
Engraulidae	<i>Engraulis australis</i>			0.8	0.1		
Gobiidae		1.0	<0.1	13.2	4.5		
	<i>Afurcagobius tamarensis</i>			10.1	3.4		
	<i>Arenigobius bifrenatus</i>			1.6	0.3		
	Gobiidae Unid.	1.0	<0.1	3.1	0.9		
Mugilidae	<i>Aldrichetta forsteri</i>			10.9	2.0	90.0	88.7
Percidae	<i>Perca fluviatilis</i>			3.1	0.5		
Pleuronectidae	Pleuronectidae Unid.			3.1	0.5		
Pseudaphritidae	<i>Pseudaphritis urvillii</i>	1.0	0.1	19.4	6.7		
Retropinnidae	<i>Retropinna semoni</i>			1.6	1.6		
Sciaenidae	<i>Argyrosomus japonicus</i>			1.6	0.3	5.0	3.8
Teleostei Unid.		31.3	4.7	22.5	11.6	2.5	1.9
Crustacea (Malacostraca)		69.8	74.7	44.2	32.8	5.0	3.8
Amphipoda	Amphipoda (all)	51.0	37.0	5.4	8.9		
Decapoda							
Brachyura	Crab unid.	5.2	0.2	0.8	0.1		
Grapsidae	<i>Paragrapsus gaimardii</i>	6.3	0.2	33.3	13.2	2.5	1.9
Hymenosomatidae	<i>Amarinus lacustris</i>	7.3	0.2				
Portunidae	<i>Ovalipes australiensis</i>					2.5	1.9
Caridea (Palaemonidae)	<i>Macrobrachium</i> sp.	2.1	0.1				
Unid. Decapod (prawn)				0.8	0.1		
Mysidacea	Mysidacea (all)	50.0	37.0	8.5	10.4		
Polychaeta		21.9	1.2	1.6	0.3		
Nereididae		19.8	1.0	0.8	0.1		
	<i>Australonereis ehlersi</i>	1.0	<0.1				
	<i>Simplisetia aequisetis</i>	8.3	0.3				
	Nereid Unid.	11.5	0.7	0.8	0.1		
Nephtyidae	<i>Aglaophamus australiensis</i>			0.8	0.1		
Polychaeta Unid.	Polychaeta Unid.	3.1	0.1				
Insecta	Lepidoptera (moth)			0.8	0.1		
Macroalgae	Macroalgae Unid.	1.0	N/A	3.9	N/A		
Unidentified matter	Unidentifiable matter	4.2	N/A				
<i>n</i> =		96		129		40	

* Macroalgae and unidentified matter were included for %F, but not applicable for %N.

Appendix 6. Pictures of some teleost prey items found in the stomachs of mulloway: yellow-eye mullet, *Aldrichetta forsteri* (top left), mulloway, *Argyrosomus japonicus* (top right), congolli, *Pseudaphritis urvillii* (middle left), Tamar goby, *Afurcagobius tamarensis* (middle right), smallmouthed hardyhead, *Atherinosoma microstoma* (bottom left) and sandy sprat, *Hyperlophus vittatus* (bottom right). Blue scale bar is in cms, silver ruler is in mms and white grids are 5 mms.



Appendix 7. Percentage contribution of prey items by number (%N) and frequency (%F) to total number and frequency for small (<80 mm) and large (>80 mm) congolli in the Coorong and Lower Lakes regions of the Coorong. Regions (Murray Estuary and North Lagoon) are not separated for the Coorong.

Family (or higher)	Prey items	Area		Coorong				Lower Lakes	
		Size class (mm)		<80		>80		>80	
		%N	%F	%N	%F	%N	%F		
Teleostei				0.7	16.4	7.2	30.8		
Atherinidae	<i>Atherinosoma microstoma</i>			0.1	2.2				
Eleotridae	<i>Philypnodon grandiceps</i>					1.2	7.7		
Gobiidae				0.1	3.0				
Poecilidae	<i>Gambusia holbrooki</i>					1.2	7.7		
Teleostei Unid.	Teleost Unid.			0.3	9.0	4.8	23.1		
	Eggs			0.1	3.0				
Crustacea		91.2	86.0	93.1	80.6	68.7	84.6		
Branchiopoda				0.1	0.7	7.2	30.8		
Cladocera	<i>Daphnia</i>			0.1	0.7	1.2	7.7		
	cysts					6.0	23.1		
Malacostraca		67.8	83.5	92.9	80.6	60.2	84.6		
Amphipoda		67.2	83.5	85.7	76.1	25.3	38.5		
	Gammaridea	65.7	78.5	43.2	59.7	25.3	38.5		
	<i>Melita</i>	1.3	13.2	42.4	24.6				
	Senticaudata	0.1	0.8	0.1	0.7				
Decapoda		0.2	0.8	0.2	3.7	34.9	61.5		
Astacoidea									
(Parastacoidea)	<i>Cherax destructor</i>					1.2	7.7		
Brachyura		0.2	0.8	0.2	3.7				
	Crab unid.			0.1	2.2				
Grapsidae	<i>Paragrapsus gaimardii</i>	0.1	0.8	0.1	1.5				
	(total)								
	<i>Paragrapsus gaimardii</i>			0.1	0.7				
	(adult)								
	<i>P. gaimardii</i> (post-larvae)	<0.1	0.8	0.0	0.7				
	<i>P. gaimardii</i> (megalopa)	<0.1	0.8						
Hymeno-									
somatidae	<i>Amarinus lacustris</i> (post-	0.1	0.8						
	larvae)								
Caridea (Atyidae)	<i>Paratya australiensis</i>					33.7	53.8		
Mysidacea		0.4	5.0	7.0	31.3				
	Mysidacea (adult)	0.4	5.0	5.5	31.3				
	Mysidacea (post-nauplii stage)			1.6	2.2				
Maxillopoda (Copepoda)		23.3	14.0	0.1	1.5				
	Calanoida	0.9	4.1	0.1	1.5				
	Cyclopoida	17.1	4.1						
	Copepoda Unid.	5.4	7.4						
Ostracoda		0.1	0.8						
Isopoda		0.2	1.7						
Crustacea Unid.				0.1	1.5	1.2	7.7		
Insecta				0.1	0.7	20.5	69.2		
Diptera				0.1	0.7	7.2	30.8		
Chironomidae				0.1	0.7	4.8	15.4		
	Chironomidae unid. larvae			0.1	0.7	3.6	15.4		
	Chironomidae unid. pupae					1.2	7.7		
	<i>Tanytarsus</i> sp. pupae								
Stratiomyidae	Stratiomyidae (larvae)					1.2	7.7		

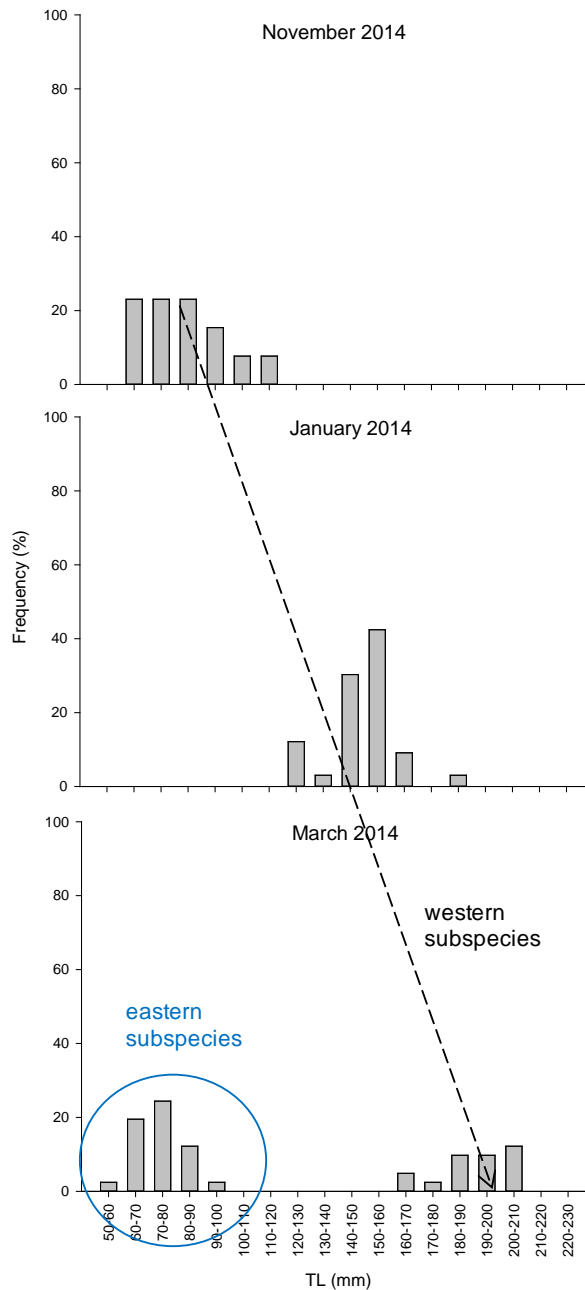
Family (or higher)	Prey items	Area		Coorong				Lower Lakes	
		Size class (mm)		<80		>80		>80	
		%N	%F	%N	%F	%N	%F		
Unid. Diptera	Unid. Diptera					1.2	7.7		
Hemiptera						6.0	30.8		
Corixidae	<i>Sigara</i> sp.					3.6	23.1		
Fulgoroidea	Fulgoroidea unid.					1.2	7.7		
Hebridae	<i>Hebrus axillaris</i>					1.2	7.7		
Notonectidae	<i>Anisops</i> sp.								
Lepidoptera	Lepidoptera (moth)								
Ordonta						7.2	23.1		
Aeshnidae	Aeshnidae unid. (nymph)					1.2	7.7		
Coenagrionidae	<i>Ischnura heterosticta</i> (nymph)					4.8	7.7		
Trichoptera	<i>Ecnomus</i> sp.					1.2	7.7		
Bivalvia	<i>Arthritica</i>			0.1	0.7				
Gastropoda		0.1	1.7	0.6	4.5	3.6	15.4		
Hydrobiidae		<0.1	0.8	0.4	2.2				
Gastropoda Unid.		<0.1	0.8	0.2	3.0	3.6	15.4		
Polychaeta		8.3	31.4	5.1	48.5				
Capitellidae		6.9	10.7	2.2	6.0				
Nereididae		1.4	24.8	2.9	47.0				
	<i>Australonereis ehlersi</i>	<0.1	0.8	0.5	9.0				
	<i>Simplisetia aequisetis</i>	<0.1	0.8	0.4	6.7				
	Nereid Unid.	1.3	24.0	2.0	39.6				
Oligochaeta		0.3	2.5	0.1	0.7				
Sipuncula		0.1	0.8						
Plant/Macroalgae*	Plant/Macroalgae Unid.							3.0	7.7
Unidentified matter*								0.7	
Unidentified				0.4	3.7				
<i>n</i> =			121		134			13	

Appendix 8. Stomach-content sample sizes of small (<80 mm TL) and large (>80 mm TL) congolli, divided by sites and months. B19 = Beacon 19, BCM = Boundary Creek Mouth, BCS = Boundary Creek Structure, TAU = Tauwitcherie, MP = Mark Point, LP = Long Point, NM = Noonameena.

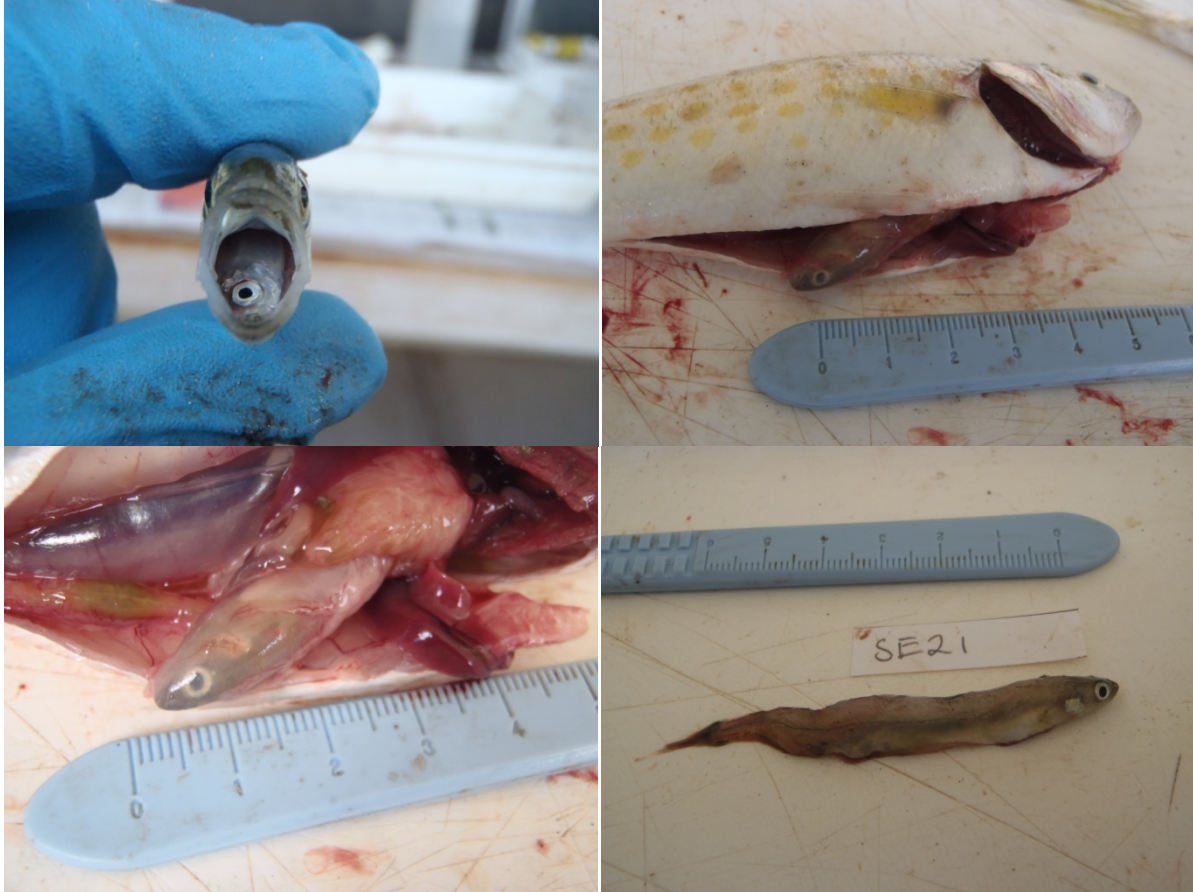
Size/Month	Site						
	B19	ME			NL		
		BCM	BCS	TAU	MP	LP	NM
Small							
Nov	4		7	5	1	5	3
Jan	5			19	6	14	12
Mar		4				20	16
Total	9	4	7	24	7	39	31
Large							
Nov				29	7	45	7
Jan				2	2	6	20
Mar					1	5	10
Total	0	0	0	31	10	56	37

Appendix 9. Mean (\bar{x}) Australian salmon total length (mm) \pm standard deviation (S.D.) for each month, separated by species. Size frequencies for each month are also provided below showing the progression of the western species cohort from November through to March. The eastern species cohort appeared in March.

Month species	Nov			Jan			Mar		
	<i>n</i>	\bar{x}	S.D.	<i>n</i>	\bar{x}	S.D.	<i>n</i>	\bar{x}	S.D.
Western	13	83.7	\pm 15.2	29	148.7	\pm 11.5	16	189.1	\pm 12.7
Eastern	0			0			20	72.0	\pm 8.0



Appendix 10. A 38 mm sandy sprat (*Hyperlophus vittatus*) protruding out of the mouth of a small juvenile salmon (78 mm, only just double its size) (top left). There was another sandy sprat found in the stomach of this same individual. A 71 mm common galaxias (*Galaxias maculatus*) that barely fits in the stomach and throat of this medium-sized juvenile salmon (170 mm) (last three pictures).



Appendix 11. Percentage contribution of prey items by number (%N) and frequency (%F) in the stomachs of Australian salmon (56–205 mm, $n = 78$). Major groups are in bold. Western ($n = 58$) and eastern ($n = 20$) Australian salmon are not separated.

Family (or higher)	Prey items	%N	%F
Teleostei		19.8	76.9
Atherinidae	<i>Atherinosoma microstoma</i>	1.7	14.1
Clupeidae	<i>Hyperlophus vittatus</i>	6.9	30.8
	<i>Nematalosa erebi</i> (post-larvae)	1.6	2.6
Galaxiidae	<i>Galaxias maculatus</i>	0.1	2.6
Gobiidae	<i>Afurcagobius tamarensis</i>	0.5	7.7
	<i>Arenigobius bifrenatus</i>	0.1	1.3
	<i>Tasmanogobius lasti</i>	0.1	1.3
	Gobiidae Unid.	0.5	7.7
Mugilidae	<i>Aldrichetta forsteri</i>	0.1	1.3
Teleostei Unid.	Teleostei Unid.	8.4	44.9
Crustacea		79.6	34.6
Malacostraca			
Amphipoda	Amphipoda	23.4	17.9
Decapoda			
Brachyura	Crab unid.	0.1	1.3
Caridea			
(Palaemonidae)	<i>Macrobrachium</i> sp.	0.1	1.3
Mysidacea	Mysidacea (all)	7.0	17.9
	Mysidacea (adult)	4.6	17.9
	Mysidacea (post-nauplii stage)	2.3	3.8
Maxillopoda (Copepoda)	Cyclopoida	49.0	10.3
Ostracoda	Ostracoda	0.1	1.3
Unid. Crustacea*	Unid. Crustacea (fragments)	N/A	3.8
Insecta		0.2	3.8
Chironomidae	<i>Tanytarsus</i> sp. pupae	0.1	1.3
Corixidae	<i>Sigara</i> sp.	0.1	1.3
Notonectidae	<i>Anisops</i> sp.	0.1	1.3
Mollusca		0.2	2.6
Donacidae	<i>Donax deltooides</i>	0.1	1.3
Hydrobiidae	Hydrobiidae	0.1	1.3
Polychaeta	Nereididae Unid.	0.3	6.4
Macroalgae*	Plant Material Unid.	N/A	1.3
Unidentified matter*	Unidentifiable matter	N/A	15.4

* Macroalgae, unidentified Crustacea (fragments) and unidentified matter are not applicable for %N.