

Food resource partitioning among three key fishery species in the Walpole-Nornalup Estuary

Kyle Stewart

Bachelor of Science, Murdoch University

Submitted for the Honours degree
of Murdoch University

2018



Declaration

I declare this thesis is my own account of my research and contains as its main content, work which has not been previously submitted for a degree at any tertiary education institution.

Kyle Stewart

Abstract

Fish dietary studies are crucial for enhancing understanding of the food web dynamics and trophic structure of ecosystems which is integral for conservation purposes. The overarching aim of this study was to assess the extent and nature of any trophic resource partitioning among three co-familial (Sparidae) fishery species (Black bream *Acanthopagrus butcheri*, Tarwhine *Rhabdosargus sarba* and Pink snapper *Chrysophrys auratus*) within an area and time of year in which they all co-occur in the Walpole-Nornalup Estuary, south-western Australia. A total of 227 fish gut samples, 186 of which contained food, were collected across three different size classes (small, 0-150 mm; medium, 150-250 mm; large, >250 mm total length) of the above species and examined for gut content composition. Muscle samples from a subset of those fish were also analysed for their stable isotope ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) composition to understand their primary energy sources and relative trophic feeding levels. Overall, dietary composition differences were small to moderate. The largest species differences occurred between the omnivorous and estuarine *A. butcheri* and the carnivorous and marine *C. auratus*, and significant ontogenetic shifts were identified only for *R. sarba* and to a lesser extent *A. butcheri*, with both species consuming more plant material with increasing size. As expected, the primary energy sources of *A. butcheri* were more freshwater/terrestrially-derived, while those of *C. auratus* had the greatest marine influences, but unexpectedly, small-medium *A. butcheri* had a notably higher trophic level than any other species or size class. The stable isotope results suggested a considerable amount of trophic overlap between *A. butcheri* and *R. sarba*. With ongoing climate change effects in south-western Australia, including further salinisation of estuaries and increased abundance of marine species, trophic competition pressures (particularly between *A. butcheri* and *R. sarba*) are expected to increase in the Walpole-Nornalup Estuary. Given the subsequent potential impacts on the productivity of these key fishery species, it is suggested that the diets, as well as the abundance and growth of these Sparidae populations, are monitored to help ensure a sustainable recreational fishing experience in the Walpole-Nornalup into the future.

Table of Contents

Abstract.....	iii
Acknowledgements.....	vi
1. Introduction.....	1
1.1 Importance of fish dietary studies and the concept of trophic niche.....	2
1.2 Resource partitioning among closely related species.....	3
1.3 Approaches to characterising fish diets	4
<i>1.3.1 Gut Content Analysis.....</i>	<i>5</i>
<i>1.3.2 Stable Isotope Analysis</i>	<i>6</i>
<i>1.3.3 Fatty acid and eDNA analysis.....</i>	<i>8</i>
1.4 Estuaries and their importance	9
1.5 Walpole-Nornalup Estuary	11
1.6 Fishery-important species in the Walpole-Nornalup Estuary, with a focus on sparids	13
1.7 Study significance and aims.....	15
2. Methods.....	17
2.1 Study area.....	17
2.2 Field methods.....	19
2.3 Laboratory methods	21
<i>2.3.1 Gut Content Analysis.....</i>	<i>21</i>
<i>2.3.2 Stable Isotope Analysis</i>	<i>22</i>
2.4 Statistical methods	23
<i>2.4.1 Gut Content Analysis.....</i>	<i>23</i>

2.4.2 Stable Isotope Analysis	25
3. Results	26
3.1 Overall characteristics of sparid gut contents	26
3.1.1 <i>Acanthopagrus butcheri</i>	27
3.1.2 <i>Rhabdosargus sarba</i>	28
3.1.3 <i>Chrysophrys auratus</i>	28
3.2 Differences in gut content composition among sparid species and size classes	30
3.3 Isotopic composition of each sparid species and size class	40
3.3.1 Trends in average $\delta^{13}C$ among species and size classes	40
3.3.2 Trends in average $\delta^{15}N$ among sparid species and size classes	41
3.4 Isotopic signatures of sparid species in relation to those of potential predators and prey	42
4. Discussion.....	44
4.1 Differences in gut content composition among <i>Sparidae</i> species.....	45
4.2 Differences in gut content composition among size classes	48
4.3 Primary carbon sources and trophic positioning of <i>Sparidae</i> species	50
4.4 Trophic partitioning	54
4.5 Management implications and directions for future research	55
4.6 Limitations and avenues for future research	57
5. Conclusions	59
6. References	61

Acknowledgements

I would firstly like to thank my honours supervisors for their tireless dedication and assistance in conducting this research. To Fiona Valesini, I am extremely grateful for all of the time you have spent reading, editing and giving me suggestions on how to best improve my work. I would also like to thank you for originally giving me the opportunity to start this honours project as I've learnt so much along the way and have finally been able to apply everything I learnt in my undergraduate degree into actually making a difference towards conserving marine life.

To Christopher Hallett, I really appreciate all of the encouragement, advice and assistance you have given me. I have a lot of respect for all of the time you have dedicated towards helping me through this project as I know you already have a lot on your plate, from juggling the supervision of several other PhD students, looking after your family and even conducting your own research. Your great work ethic and passion for conservation is really inspiring.

I would also like to thank Daniel Yeoh for helping me conduct all of my fieldwork and for battling out the many cold, rainy days we had in the Walpole. From wading through the rivers trying haul in muddy seine nets, to catching and tagging gummy sharks and smooth stingrays at 1am. I have learnt so much and really enjoyed all of the great experiences we've had.

A big thanks also to Sian Glazier for answering my many questions and helping me through all of my gut and stable isotope processing. Also, thank you to Sorch Cronin O'Reilly, Oliver Krumholz and Alan Cottingham for helping identify the many tiny partially-digested invertebrates I've come across after dissecting countless sparid guts.

On a personal level, I'd like to say thank you to my mother Leanne and girlfriend Stephanie for all the encouragement and support you guys have given me over this intense last year.

Lastly, thanks to Murdoch University and the donors of the scholarships that helped make this research possible from both Myrtle AB Lamb and the Harry Butler Institute.

1. Introduction

The teleost family Sparidae (seabreams) supports significant commercial, recreational and artisanal fisheries in numerous countries across the world, encompassing Mediterranean Europe (García-Rodríguez et al. 2006), the Middle East (Grandcourt et al. 2004), North and South America (Coleman et al. 2004, David et al. 2005), Asia (Paulin 1990), Africa (Radebe et al. 2002) and Australia (Hughes et al. 2009). Of the 159 species in the Sparidae family, three are of particular value to Australian recreational and commercial fisheries; the Black bream (*Acanthopagrus butcheri*), Tarwhine (*Rhabdosargus sarba*) and Pink snapper (*Chrysophrys auratus*) (Morison et al. 1998, Curnow 1999, Gray and Kennelly 2003, Hughes et al. 2009). These three sparids are important estuarine fishery species, especially in the case of Black bream that completes its entire life cycle within the estuarine environment, whereas the latter two marine species capitalise on estuarine food resources and shelter only during their developmental (juvenile) stages to enable rapid growth before migrating back to sea (Pollard 1994, Able 2005). Although the diets of one or more of these three species have been assessed individually in several water bodies across Australia (e.g. Paulin 1990, Sarre 1999, Peng 2003, Svensson et al. 2007, Hughes et al. 2009, Linke 2011, French et al. 2012), no studies have yet documented the dietary patterns of all three sparids within an area in which they co-occur. Understanding the diets of co-occurring and particularly co-familial species is of particular importance, as they may be more inclined to have a high degree of overlap in terms of resource use (i.e. habitats and food). The resulting inter-specific competition influences their realized niche, which in turn may affect many aspects of their life history, including growth rate,

population size, maximum size and length at maturity (Zaret and Rand 1971, Ross 1986). More generally, obtaining robust data on the diet and trophic interactions of fish species is critical for understanding their functional role in an ecosystem, which is imperative for the development of sound conservation and management plans (Schoener 1974, Hyndes et al. 1997, Platell and Potter 2001).

1.1 Importance of fish dietary studies and the concept of trophic niche

Dietary quantification studies can help determine the trophic niche of a fish species, which depicts its positioning in the food web and functional interactions with other organisms via feeding relationships (Shipley et al. 2009). Whilst many definitions exist for the term “niche”, as described by Whittaker et al. (1973), the term “trophic niche” specifically measures a species’ relationships to its prey and predators, whereas the broader term “niche” depicts not only an organism’s feeding relationships, but also the broader set of conditions, resources and interactions it requires or can make use of (Pollard 1994, Chuwen et al. 2007, Miller and Spoolman 2011). Furthermore, a species’ “ecosystem niche” specifically depicts the environmental conditions such as salinity, temperature, pH, wave action, in which a species can theoretically tolerate and survive in whereas the “realized niche” is the actual population distribution observed in nature (Elton 1927, Whittaker et al. 1973).

Together an understanding of trophic and ecosystem niche is imperative for ecosystem-based management of key fish stocks, e.g. by identifying habitats that should be protected

or enhanced to best sustain food sources for future populations (Platell and Potter 2001, Chuwen et al. 2007).

Combined information on fish dietary composition and trophic niche can be used to assess differences in diet composition to effectively model and, importantly, predict ecosystem function. Examples of this approach include the use of ecological network analysis (Ulanowicz 2004), Stable Isotope Bayesian Ellipses in R (SIBER) (Jackson et al. 2011) as well as multivariate generalised linear models (Cortés 1997). Larger scale ecosystem-encompassing models include the internationally-recognised Ecopath, Ecosim and Ecospace software (Pauly et al. 2000, Christensen and Walters 2004, Wang et al. 2012).

1.2 Resource partitioning among closely related species

The degree of functional relatedness of co-occurring species typically influences the level of trophic niche overlap, with species that share similarities in morphology (e.g. mouth structures), physiology (e.g. digestive system) and/or habitat (Ross 1986) typically having higher overlap and hence resource competition. In order to reduce inter- and intra-specific competition within an area of spatial overlap, organisms commonly employ ‘resource partitioning’, i.e. by dividing finite resources such as food and habitat to facilitate their coexistence (Roughgarden 1976, Grossman 1986, Olson et al. 1988). The competition for resources amongst similar fish species and/or different life-stages can be minimised by, for instance, the habitation of slightly different habitats, occupying preferred habitats at different times, and/or consuming different prey types (Ross 1986, Hyndes et al. 1997, Platell and Potter 2001, Halver and Hardy 2002).

Resource partitioning is often evident between fish of the same genus (congeneric) or family (confamilial), and also among different life history stages of the same species (ontogenetic shifts) (Ross 1986). For instance, in the US, two lake-dwelling sunfish of the same genus: Large bluegills (*Lepomis macrochirus*) and Pumpkinseeds (*Lepomis gibbosus*) demonstrate resource partitioning both in nature and in laboratory tank experiments whereby *L. macrochirus* will feed primarily on open-water zooplankton (*Daphnia*) and *L. gibbosus* will preferentially target vegetation-dwelling gastropods possibly to reduce resource competition (Mittelbach 1984). To minimise competition between different life history stages, the juvenile forms of many fishes typically inhabit the pelagic zone and feed on small planktonic organisms, then later transition to benthic habitats and feed on larger prey such as crustaceans and molluscs during the adult stage (Skúlason and Smith 1995, Robinson et al. 1996, Svanbäck and Eklöv 2003). The ontogenetic development of morphological traits such as feeding structures (i.e. mouth gape, shape, dentition) and those related to movement (i.e. muscle tissue growth, swim bladder formation and body shape) further help to facilitate this separation in dietary niche between life history stages (Bone et al. 1995, Hyndes et al. 1997, Xie et al. 2001).

1.3 Approaches to characterising fish diets

Dietary quantification studies typically use one or several of the following methods: Gut Content Analysis (GCA), Stable Isotope Analysis (SIA), Fatty Acid Analysis (FAA) or environmental DNA analysis (eDNA/DNA barcoding) (Smith et al. 1997, Kawaguchi and Nakano 2001, Post 2002, Chuwen et al. 2007, Carreon-Martinez et al. 2011, Kelly and Scheibling 2012, Baker et al. 2014, Jo et al. 2016). These techniques differ in several

respects in terms of the type of information they provide. One such difference is whether they elucidate dietary composition (i.e. GCA, SIA, FAA and eDNA) and/or the trophic positioning of the organism in relation to other organisms (i.e. SIA and FAA). Another key difference is the time scale over which diet is represented, with some methods only measuring the last meal (i.e. GCA and eDNA) and others reflecting diet over time scales from weeks to months (i.e. SIA and FAA) (Käkelä et al. 1993, Iverson et al. 2002, Linke 2011, Baker et al. 2014). Subsections 1.3.1-1.3.2 will provide a more detailed outline of the more commonly-used GCA and SIA, while a brief outline of FAA and eDNA is provided in subsection 1.3.3.

1.3.1 Gut Content Analysis

Gut Content Analysis involves the visual identification of prey items within a dissected stomach and/or intestine, and the use of specific indices to quantify particular dietary attributes (Cortés 1997, Baker et al. 2014). These indices include the percentage frequency of occurrence ($\%F$) of a prey category based on its presence/absence in guts across the sampled population, and the percentage numerical frequency, $\%N$ that measures the relative abundance of prey in each category within each gut. Bulk-based indices include the percentage volumetric contribution of each prey category to the total gut fullness of each individual ($\%V$), or the percentage weight contribution ($\%W$) of each prey category to the total gut weight (Cortés 1998, Sarre and Potter 2000, Hadwen et al. 2007).

Each of these indices reflects a different perspective of the most recent meal, and have accompanying advantages and disadvantages. For instance, while the $\%F$ is great at determining the most frequently preyed upon guts for a particular species, it is less

informative in describing the diet of an individual fish as it relies on presence/absence data. Whilst the %N has a much finer resolution and can quantitatively describe diet on both the scale of individual fish and species, it has strong biases associated with the counting of fragmented and heavily digested prey items and also the quantification of plant matter such as algae and seagrass (Baker et al. 2014). Alternatively, the %V and %W reflect the bulk of the most ‘preferred’ prey, but have biases associated with the visual estimation of prey volume (Baker et al. 2014).

One approach for down-weighting the biases of individual indices is to use a combination (e.g. %F, %N and %W) to calculate an Index of Relative Importance (IRI) for each prey category (Pinkas et al. 1971, Cortés 1997). However, criticism of the IRI method have been made by multiple authors (Berg 1979, Macdonald and Green 1983, Bowen 1996, Hansson 1998), with the latter arguing that it is strongly influenced by the taxonomic resolution of each prey categories.

Gut Content Analysis has traditionally been used as the sole means of determining fish diets since it is simple to conduct, does not require advanced technology or equipment, and yields quantitative information on dietary composition. However, as outlined above, it has several limitations, including only measuring the recent feeding events and the difficulties in accurately identifying readily-digestible prey items (Polis 1984, Michener 1994, Hansson et al. 1997).

1.3.2 Stable Isotope Analysis

Stable Isotope Analysis utilises a mass spectrometer to extract stable isotopes, which are alternate forms of key elements such as carbon, nitrogen and sulphur, from the tissues of

a target organism to provide information on its dietary composition, trophic position and primary energy source (Tieszen et al. 1983, Katzenberg 2007). The isotopic ratio of $\delta^{13}\text{C}$ (i.e. the ratio between the abundance of the stable C^{13} isotope and the lighter C^{12} isotope) varies substantially between different primary producers (e.g. C_3 vs C_4 plants), but changes little with increasing trophic level (Layman et al. 2007). Positive (‘saturated’) $\delta^{13}\text{C}$ values indicate a marine-sourced diet, whereas negative (‘depleted’) values indicate a terrestrial or freshwater-derived diet (DeNiro and Epstein 1981, Peterson and Fry 1987, Post 2002, Fry 2006). In contrast, the isotopic signature for $\delta^{15}\text{N}$ (i.e. the ratio between the abundance of N^{15} and the lighter N^{14} isotope) changes predictably and accumulates along trophic pathways, making it a useful indicator of the trophic level of an organism (DeNiro and Epstein 1978, Fry 2006, Traugott et al. 2013). $\delta^{15}\text{N}$ is substantially lower in organisms with low protein diets (e.g. primary consumers) than those with high protein diets (e.g. secondary and tertiary consumers), due to higher rates of N^{15} assimilation relative to excretion in the latter (DeNiro and Epstein 1981, Post 2002, McCutchan et al. 2003).

The isotopic signatures of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ can differ substantially both within and among species. Scatterplots (‘biplots’) reflecting the values of both of these signatures in each consumer and producer of interest are commonly used in dietary studies, enabling the visual estimation of trophic levels and key energy sources. However, these stable isotope biplots can only provide a qualitative measure of trophic level and marine influence with quantitative techniques such as Bayesian mixing models being required for formal statistical testing and the determination of trophic niche (Busst and Britton 2016).

1.3.3 Fatty acid and eDNA analysis

Two alternative means of measuring fish dietary composition are fatty acid analysis and eDNA analysis, with each having their own specific advantages and limitations. The first of these methods utilises fatty acids as trophic markers (FATM) which can be extracted from predator tissue samples to determine predator-prey relationships (Cook 1991, Iverson et al. 2002, Dalsgaard et al. 2003, Iverson et al. 2004). Since fatty acids pass reliably from primary producers to consumers up the food chain, they provide an indication of key food sources over time periods of months to years (St John and Lund 1996, Kirsch et al. 1998, Auel et al. 2002). To date, however, fatty acids have been used mainly as a qualitative food web marker (i.e. presence/absence of prey items), with quantitative dietary source measurement (i.e. statistically testable) remaining difficult as this requires an understanding of how fatty acids differ in a given ecosystem and statistical models that relate the predator signature to a mixture of possible prey signatures (Iverson et al. 2004). However, if conducted accordingly, FAA can provide very high-resolution indicators of trophic linkages, more so than the previously described SIA technique due to the vast array of lipids in primary producers (Bobbie and White 1980, Dalsgaard et al. 2003, Iverson 2009, Traugott et al. 2013). Fatty acid analysis can additionally be used to trace the origin of dietary sources and is also capable of distinguishing and characterizing marine fish and invertebrates with regards to different size groups, potential diets and habitats (Meziane and Tsuchiya 2000, Abdulkadir and Tsuchiya 2008). The constraints of the method include that fatty acids are not unique to particular organisms, and the fact that fatty acid stability largely depends on the organisms' metabolic strategy and condition

(Dalsgaard et al. 2003). Because of the limitations associated with this method, it is typically used in conjunction with a more quantitative measure of diet such as GCA.

Environmental DNA analysis involves examining DNA from homogenised gut or faecal samples and extracting short genetic markers or “barcodes” which can be compared against a DNA library such as GenBank for prey identification (Group et al. 2009, Ward et al. 2009, Carreon-Martinez et al. 2011). Environmental DNA analysis is advantageous due to its potential to identify prey to a high resolution (up to species or sub-species level) from degraded samples, which is largely not possible with visual methods such as GCA. Its ability to be used on faecal samples also allows for non-invasive dietary identification (Jarman et al. 2002, Deagle et al. 2005). However, eDNA analysis also has its limitations. For example, it requires a comprehensive library of prey DNA signatures to reliably interpret consumer dietary composition (Harris 2003, Ward et al. 2009), is prone to contamination with non-target DNA (Binladen et al. 2007, Deagle et al. 2009, Pompanon et al. 2012), does not provide a quantitative understanding of dietary composition, is expensive and, like GCA, only reflects the most recent meals (Jo et al. 2016).

1.4 Estuaries and their importance

Estuaries are the transitional waters between marine and freshwater environments. Their unique physico-chemical conditions are highly dynamic and are influenced by various climatic and hydrodynamic forces, such as rainfall, tidal flow, streamflow and wave energy (Wolanski and Elliott 2015, Raimonet and Cloern 2017). Estuarine water conditions thus vary markedly over daily, seasonal and interannual timescales, as well as

over horizontal and vertical spatial gradients (Roy et al. 2001, Whitfield and Elliott 2011, Potter et al. 2015). Estuaries are often extremely productive ecosystems, attributed largely to the photosynthetic activity of phytoplankton and peri-phyton and the flow of organic carbon from their catchments (Correll 1978, McLusky et al. 2004, Paerl 2006). Consequently, they typically sustain abundant faunal communities (Heip et al. 1995, Costanza et al. 2007, Elliott and Whitfield 2011).

Estuaries provide ‘nurseries’ for the juveniles of many marine and some freshwater fish species (marine and freshwater estuarine-opportunists, respectively), migratory routes between the sea and riverine environments for various ‘diadromous’ species, as well as the sole environment for various species able to complete their life-cycle in these dynamic environments (estuarine residents or marine and estuarine species) (Potter and Hyndes 1994, Sarre et al. 2000, Potter et al. 2015). The fish nursery function provided by many estuaries reflects not only their immense productivity, but also their protected shallow waters, often warmer temperatures, as well as their abundance and diversity of both food sources and habitats (Pollard 1994, Able 2005).

From a societal perspective, the highly productive nature of estuaries supports many industries such as fishing and ecotourism, and these systems represent important sources of food and income for local populations (Lenanton and Potter 1987, Blaber 2008, Whitfield 2016). For instance, in the US, estuarine fish accounted for 46% of the total catch by commercial fisherman from 2000-04 and approximately 80% of all recreational landings (Lellis-Dibble et al. 2008). In Australia, around 75% of the national commercial fishery catch comprises species that spend at least part of their life within an estuary, and

over 35% of all recreational fishing effort occurs in estuarine systems (Henry and Lyle 2003, Creighton et al. 2015).

The high degree of human activity within and surrounding estuaries does take its toll on the health of these ecosystems however. For example, many estuaries have been dredged and modified to accommodate shipping and boating, whilst industrial and agricultural practices in surrounding catchments have led to various harmful downstream impacts, such as excessive nutrient and organic matter loading (with subsequent negative effects on water quality through algal blooms and anoxia), non-nutrient pollution and sedimentation (Kennish 2002, Paerl 2006, McCluskey and Lewison 2008, Elliott et al. 2016). Fishing also places direct stress on estuarine fish populations, with overfishing causing potentially dramatic changes to the abundance, size and/or age structure of targeted species, from which they may take many years to recover, if at all (Blaber et al. 2000, Chuwen et al. 2009, Cottingham et al. 2014, Cottingham 2016a, Cottingham 2016b, Whitfield 2016).

1.5 Walpole-Nornalup Estuary

The Walpole-Nornalup Estuary, located along the south coast of Western Australia (WA), is one of only seven estuaries in the broader south-western Australian region that remains permanently open to the sea (Yeoh 2018). It is also one of the most biodiverse estuaries along the south coast, and is considered to be largely unmodified from its pristine state (NLWRA 2002). Surrounded by dense native forest, the Walpole-Nornalup Estuary supports many ecologically-significant values, including diverse fish communities, marine mammal species such as the Indo-Pacific bottle nose dolphin (*Tursiops aduncus*)

and nesting grounds for native bird species such as the Western osprey (*Pandion haliaetus*), as well as various social values including tourism and recreational fishing (Deeley 2001, Semeniuk et al. 2011). In recognition of these values, the estuary was gazetted as the Walpole-Nornalup Inlets Marine Park (WNIMP) in 2009. The estuary is fringed by a town of only 400 residents (ABS, 2016), but which increases substantially during peak tourist season in summer, many of whom come to the region to fish.

Although the Walpole-Nornalup Estuary is relatively small in size compared to other estuaries in Australia's south-west, it has some of the highest recreational fishing pressures across the region (Smallwood and Sumner 2007). For instance, the annual recreational harvest of *A. butcheri* in the Walpole-Nornalup Estuary (i.e. 14.8 tonnes) is comparable the densely-populated Swan-Canning System which is approximately 16 tonnes (Smith and Smith 2006). In terms of total annual catch statistics for the three sparid species examined, recreational fishing effort is typically higher for *A. butcheri* than both *C. auratus* and *R. sarba*. For instance, from 2002 to 2003, the estimated annual recreational fishing effort for *A. butcheri* in nearshore marine and estuarine environments in the South Coast Bioregion was 23.3 tonnes, which was far greater than that of *C. auratus* at 2.6 tonnes and *R. sarba* at 0.5 tonnes (Smallwood and Sumner 2007).

Furthermore, the Walpole-Nornalup Estuary is located in a global climate change hotspot and is experiencing unprecedented declines in winter rain fall and increasing annual mean temperatures. Since the 1970s the mean air temperature has risen by an average of 1 °C, whilst an estimated decline in rainfall of 15-20% has caused the overall freshwater flow to the Walpole-Nornalup Estuary to decrease by more than half (Petrone et al. 2010, Barron et al. 2012, Silberstein et al. 2012, Hope et al. 2015). These changes have led to

several estuaries in the region becoming progressively more marine (Valesini et al. 2017, Hallett et al. 2018), which is likely to impact the trophic niche and distribution patterns of fish species that use estuaries through changes in their abundance (e.g. based on their affinities for marine conditions) and that of their food sources. Furthermore, the accompanying changes to rainfall volume, timing and intensity will likely cause many intermittently-open estuaries on the south-west coast to close for longer periods, which may result in the few permanently-open systems such as the Walpole-Nornalup Estuary becoming even more important as nursery areas for marine fish species (Hoeksema and Potter 2006, Gillanders et al. 2011).

1.6 Fishery-important species in the Walpole-Nornalup Estuary, with a focus on sparids

Over 20 commercial and/or recreational fishery species have been recorded in the Walpole-Nornalup Estuary, including the Australian herring (*Arripis georgianus*), King George whiting (*Sillaginodes punctatus*), Tailor (*Pomatomus saltatrix*), Estuary cobbler (*Cnidogobius macrocephalus*) and the Southern bluespotted flathead (*Platycephalus speculator*) (Potter et al. 1996, Smallwood and Sumner 2007, Fletcher and Santoro 2012, Ryan et al. 2015, Yeoh 2018). Among these species, three of the most recreationally-targeted in the estuary are from the Sparidae family, namely the Black bream (*Acanthopagrus butcheri*), Pink snapper (*Chrysophrys auratus*) and Tarwhine (*Rhabdosargus sarba*).

Of the three sparid species in the Walpole-Nornalup, *A. butcheri* is the only one that completes its life cycle within the estuarine environment. *Chrysophrys auratus* and

R. sarba are considered marine-estuarine opportunists, and thus utilize the estuary only during their juvenile phase (Potter and Hyndes 1999). After maturity, all three sparids frequently occupy a benthic-pelagic positioning in the water column and therefore, are likely to feed on prey from both the benthos and surface waters of estuarine systems (Antonucci et al. 2009, Santini et al. 2014). Sexual maturity is reached between 150-210 mm for *R. sarba* in WA and between 150-160 mm for *A. butcheri* in the Walpole-Nornalup Estuary (Sarre and Potter 1999, Hughes et al. 2009, Cottingham et al. 2018). *Chrysophrys auratus* matures at much larger lengths than the previously discussed sparids at approximately 600 mm for females and 586 mm for males (L_{50}) on the south coast of WA (Wakefield 2006). *Acanthopagrus butcheri* and *R. sarba* share the same Minimum Legal Length (MLL) of capture at 250 mm, whereas the MLL for *C. auratus* is a much larger 410 mm; individuals of which are very rare in the Walpole-Nornalup Estuary as adults will typically leave the system once they reach maturity (DPIRD 2018, Yeoh 2018).

Currently considerable information exists on the diets of these species in estuarine and/or marine waters. For instance, *Rhabdosargus sarba* feeds predominately on crustaceans, molluscs, macrophytes and sediment-dwelling invertebrates, but has been found to become herbivorous with increasing size (Peng 2003). In the Walpole-Nornalup Estuary, *A. butcheri* has been found to share similar feeding trends to *R. sarba*, feeding on small polychaetes and amphipods during the juvenile stages of development (<99 mm) and progressively feeding on larger volumes of seagrass (*Ruppia megacarpa*) throughout maturity (Sarre et al. 2000). From previous studies in WA estuaries, it has been found that *A. butcheri* is a highly ecosystem plastic species in the sense that their diet is very variable depending on the conditions of the prevailing ecosystem (Sarre 1999, Sarre et al. 2000,

Chuwen et al. 2007). For example, *A. butcheri* in the Wellstead Estuary fed primarily on macroalgae (*Chaetomorpha* spp.) and tube-dwelling amphipods, whereas seagrass (*Ruppia megacarpa*) and teleosts were consumed in much higher volumes by those in the Walpole-Nornalup Estuary (Sarre et al. 2000). *Chrysophrys auratus* however, tend to remain primarily omnivorous throughout life, but have been documented to feed on larger brachyuran crabs, teleosts and echinoids at larger sizes, and hence tend to be more carnivorous than the aforementioned sparids (Colman 1972, Russell 1983, French et al. 2012).

1.7 Study significance and aims

A recent acoustic fish-tracking study by Yeoh (2018) highlighted a considerable degree of spatial and temporal overlap in the distributions of the above three sparid species in the Walpole-Nornalup Estuary, namely in the eastern Nornalup Inlet and Frankland River mouth (Figure 2.1) during autumn when salinities throughout much of the system remain largely marine. Such a degree of overlap, and the fact that all of these species occupy a benthic-pelagic position in the water column and are considered to some degree to be opportunistic feeders (Antonucci et al. 2009, Santini et al. 2014), raises the question of how these confamilial species partition their food resources in this estuarine system. Whilst research into the diets of *A. butcheri* has been undertaken in the Walpole-Nornalup Estuary (Sarre et al. 2000, Sarre and Potter 2000), no research has been conducted on the diets of *R. sarba* or *C. auratus* in the system, nor has the degree of resource overlap been assessed between the three sparids in any estuarine environment throughout Australia. Information on the diet and trophic interactions of these sparids is essential in

understanding their functional role in the Walpole-Nornalup Estuary, and is necessary for the effective management of this strong recreational fishery (Schoener 1974, Hyndes et al. 1997, Platell and Potter 2001). This study will aid in filling the knowledge gap as to how sparids partition their food resources within the Walpole-Nornalup Estuary, as well as contribute to wider understanding of confamilial resource partitioning among estuarine fish species. The specific aims of this study are as follows, with accompanying hypotheses shown in italics.

1. To use Gut Content Analysis to assess the extent of any differences in dietary composition amongst *A. butcheri*, *R. sarba* and *C. auratus* within the area and season of greatest overlap in the Walpole-Nornalup Estuary. *It is hypothesized that the most dietary overlap will occur between A. butcheri and R. sarba with these species being largely omnivorous, whereas C. auratus is anticipated to have a more carnivorous diet.*
2. To evaluate any differences in dietary composition amongst three size classes (0-150 mm, 150-250 mm and >250 mm total length) of the sparid species. *It is hypothesized that significant ontogenetic shifts will occur between the smallest and largest size classes within each sparid species. Furthermore, the greatest overlap will occur between the smallest size classes of the various species, with less overlap occurring between the largest size classes, which are likely to have more specialised morphological traits and habitat preferences than their juvenile counterparts.*

3. To determine the trophic level and primary energy sources (marine or freshwater/land derived) of each of the above sparid species using stable isotope analysis, to gain insights into their positioning on the food web in the Walpole-Nornalup Estuary. *It is hypothesized that C. auratus will be at a higher trophic level and have a more marine-derived diet than the other sparids, whilst A. butcheri and R. sarba will lie at a similar trophic level and have a more terrestrial-derived diet.*

2. Methods

2.1 Study area

The Walpole-Nornalup Estuary is located on the south coast of Western Australia (35.005° S, 116.725° E). It has two basins, the Walpole Inlet (100 ha) and larger Nornalup Inlet (1300 ha) that are fed by three main tributaries, the Frankland, Deep and Walpole rivers (Figure 2.1). The Walpole-Nornalup Estuary maintains a permanently-open connection to the sea, with its narrow entrance (~200 m wide) being sheltered from marine sand deposition by steep granite hills (Pen 1997, Hodgkin and Hesp 1998, Semeniuk et al. 2011). Overall, the main basins are quite shallow with an average depth of approximately 1 m, with the deepest points (~6 m) located in their centres, whilst the depth of the three rivers are approximately 2 m on average but can reach up to 5 m in certain areas (Hodgkin and Clark. 1999).

Both basins have little structural complexity and typically have bare sand or silty mud substrates, with isolated areas of seagrass and rocky reef. The tidal rivers have substantially more structure due to fallen trees and the dense fringing vegetation (Huisman et al. 2011, Semeniuk et al. 2011).

The majority of freshwater flow entering the estuary is provided by the Frankland River (~ 60%), whereas the smaller Deep and Walpole Rivers provide 30 and 5% of flow, respectively (Hodgkin and Clark 1988, Brearley 2005, Semeniuk et al. 2011). The basins typically have a strong marine influence, with salinities in the deeper waters remaining close to that of sea water for most of the year (i.e. 35 ppt), with heavy rainfall during the winter months causing the rivers to be primarily fresh throughout (Hodgkin and Clark 1988, Brearley 2005, Semeniuk *et al.* 2011). The Frankland River in particular, is secondarily salinized and because of such experiences further salinisation from agriculture and urbanisation (Brearley 2005, Semeniuk *et al.* 2011).

The Walpole region experiences a temperate climate that is microtidal (<0.9 m tidal range) and receives 1300 mm of rainfall per year, which is nearly twice that of Perth, the capital city of WA, at ~734 mm (Brearley 2005, Water Corporation WA 2018, Semeniuk et al. 2011). The catchment area for the Walpole-Nornalup Estuary is the second largest on the south coast of WA at 5,785 km², with the majority of the catchment area comprising of undulating forests and low-lying peat swamp (Brearley 2005). Whilst the majority of catchment for the Deep and Walpole Rivers remains as untouched national parkland, the northern end of the Frankland river catchment have been extensively cleared for agricultural purposes which include cereal cropping, sheep farming, and the cultivation of vegetables (Brearley 2005).

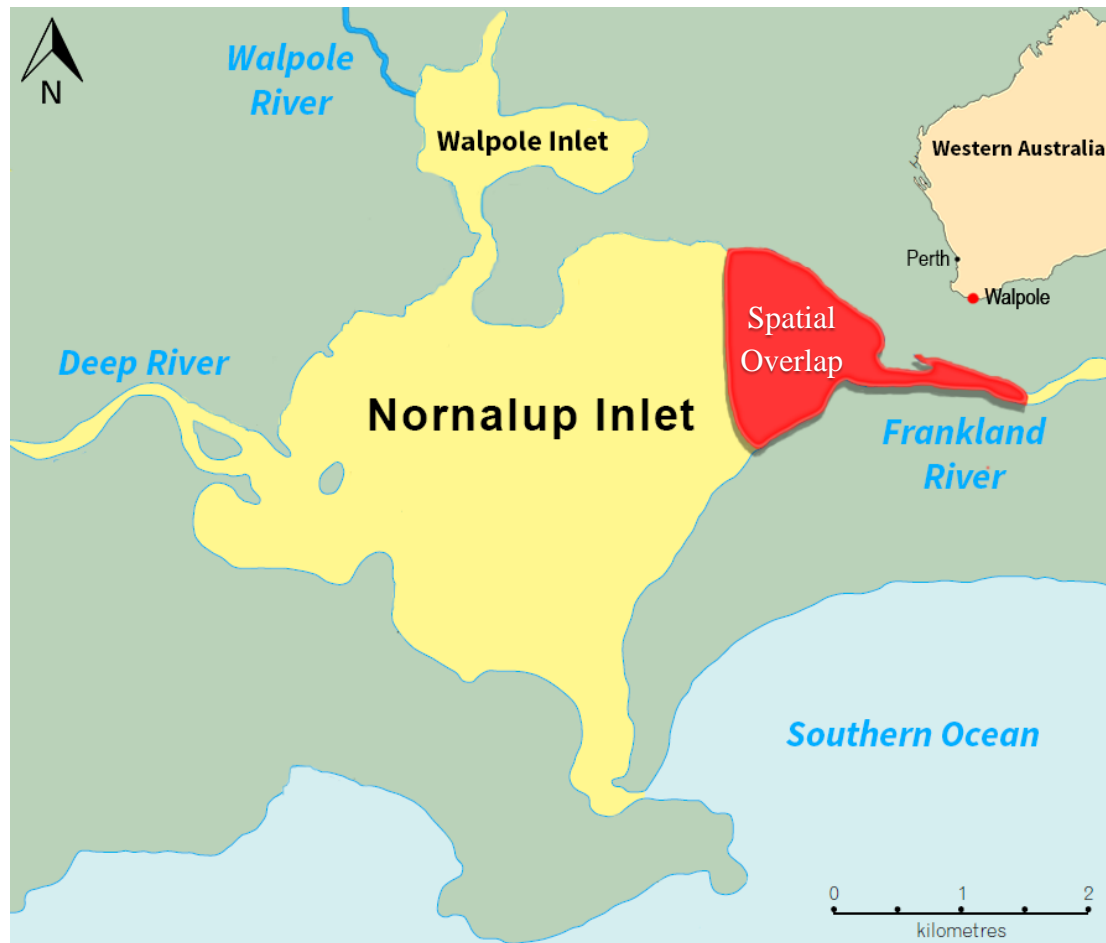


Figure 2.1. Map of the Walpole-Nornalup Estuary, showing the area (red zone) in which the overlap of *Acanthopagrus butcheri*, *Rhabdosargus sarba* and *Chrysophrys auratus* was found to be the strongest by Yeoh (2018). Inset shows the location of the estuary in Western Australia. Map was modified from www.parks.dpaw.wa.gov.au.

2.2 Field methods

Fish sampling was conducted in the north-eastern region of the Nornalup Inlet and lower Frankland River (see red zone on Fig. 2.1) during the Austral autumn (May and early June) of 2018. This sampling area and time of year was shown by Yeoh (2018) to represent the highest degree of spatio-temporal amongst the three sparid species of interest.

Individuals of the three target species were sampled using various fishing techniques, including sunken multi-mesh gillnets (160 m in length, 2 m in height with mesh diameters of 38-127 mm) in deeper waters (>1.5 m deep), beach seine nets (21.5 m in length, 1.5 m in height with mesh sizes of 3-25 mm) in the shallows (<1.5 m deep), and rod and line in targeted areas. Seine nets were deployed in the morning from 0600 to 0900 h in shallow sandy locations and hauled onto the shoreline of the sampling region, whereas the gill nets were deployed at night from 2000 h and retrieved three hours later. For rod and line fishing, artificial lures were mainly used to reduce the contamination of fish stomach contents with bait. However, to better attract Pink snapper in particular, river prawns (Malacostraca) and sardines (*Sardinops sagax*) were also used as bait, then were removed from the gut contents prior to analysis (see below). All retained sparids were immediately euthanized in an ice slurry following capture, then were later frozen after stable isotope samples were acquired. Wherever possible, all individuals of non-target species that were caught during sampling were immediately returned alive to the water.

For each of the target species, individuals spanning the full size range typically present in south-western Australian estuaries (i.e., 0-350 mm) (Potter et al. 1993, Potter and Hyndes 1994) were sought during sampling. Fish were then assigned to one of three size classes, namely 0-150 mm, 150-250 mm and >250 mm in total length (hereafter referred to as small, medium and large fish, respectively). The smallest size category represents sexually immature individuals of all three sparid species, whereas the medium size category encompasses individuals that are either just reaching sexual maturity or are sexually mature in the case of *A. butcheri* and *R. sarba* (i.e. ~150-160 mm and 150-210 mm respectively; Cottingham et al. 2018, Hughes et al. 2009). *Chrysophrys auratus* does

not mature until it reaches lengths of ~ 600 mm (Wakefield 2006). The larger size category was chosen to represent individuals that are above the minimum legal length of capture (MLL) for *A. butcheri* and *R. sarba* (250 mm) (DPIRD, 2018). The MLL for *C. auratus* is 410 mm (DPIRD, 2018). A sample size of 30 individuals from each respective size class was originally aimed to be analysed but due to limited sampling time and the success of fishing techniques, the actual examined sample size varied slightly amongst size classes (three and zero *R. sarba* and *C. auratus* captured >250 mm).

2.3 Laboratory methods

2.3.1 Gut Content Analysis

The total length (to the nearest mm) and weight (to the nearest g) of each fish was initially measured before the guts (stomach and intestine) were then removed and stored in 70 % w/v ethanol. Gut Content Analysis was conducted by first estimating gut fullness by ranking the gut from 1-10 in terms of how much volume identifiable prey items took up in reference to the total volume of the gut. Prey items were then identified to the lowest possible taxonomic resolution under a dissecting microscope using various taxonomic keys for each respective prey type including Crustacea.net, POLiKEY as well as various algal taxonomy books (Huisman et al., 2006).

Three indices were chosen to quantify gut contents, which each reflected a different perspective of dietary composition (see subsection 1.3.1). These included the percentage frequency of occurrence (%F), volumetric contribution (%V) and the weight contribution (%W) of each prey category. The %F was calculated for each prey category by dividing the total amount of guts the item appeared in by the total number of guts in each of the

examined sparid size classes. The %V was determined by placing the contents of each gut onto a petri dish marked with a 10x10 mm grid, sorting them based on prey type, then dividing the number of squares occupied by each prey type by the total number of occupied squares. Percentage weight contribution (%W) was obtained by weighing each prey category within a single gut and dividing this value by the total weight of all combined items in that gut.

2.3.2 Stable Isotope Analysis

Approximately 1-2 g of dorsal muscle tissue was removed from the upper region directly posterior to the head and before the beginning of the dorsal fin. Muscle tissue was removed from fresh fish using a sterile scalpel, then placed into a 2 ml Eppendorf container and frozen at -20 °C for later processing. Tissue samples were taken from eight individuals of each species and size class, with the exceptions of the >250 mm size class for both *R. sarba* and *C. auratus*, for which only three and no fish were caught, respectively.

These tissue samples were dried in a drying oven at 60 °C for approximately 48 hours until they were completely dry and firm. The samples were then processed through a TissueLyser to convert them into a fine powder suitable for stable isotope analysis (Skrzypek 2013). The powdered samples were weighed into 5x8 mm tin capsules (0.5 mg excluding capsule weight) and were sent to Edith Cowan University, Western Australia, to be analysed for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ signatures using a continuous flow-isotope 10 ratio mass spectrometer. Nitrogen isotope raw data was normalised to the international scale based on multi-point linear regression using IAEA-N2 ($\delta^{15}\text{N}$ vs Air = +20.3‰), IAEA-CH600

($\delta^{15}\text{N}$ vs Air = 1.0 ‰) and USGS40 ($\delta^{15}\text{N}$ vs Air = -4.5‰). Carbon isotope raw data was normalised to the international scale based on multi-point linear regression using IAEA-CH6 ($\delta^{13}\text{C}$ vs VPDB = -10.449‰), IAEA-CH600 ($\delta^{13}\text{C}$ vs VPDB = -27.771‰), IAEA-LSVEC ($\delta^{13}\text{C}$ vs VPDB = -46.6‰) and USGS40 ($\delta^{13}\text{C}$ vs VPDB = -26.389‰).

2.4 Statistical methods

2.4.1 Gut Content Analysis

All of the following statistical analyses were conducted using the PRIMER-E v7 multivariate statistical software (Clarke and Gorley 2015) with the PERMANOVA+ add-on module (Anderson et al. 2008). Only “full guts”, i.e. those that have at least one identifiable prey item were considered in GCA, with items such as sand, scales being excluded from statistical testing, as they provide little to no nutritional value. Prior to analysis, the data for %V and %W of each prey type in each gut sample were initially square-root transformed to reduce skewness and better balance the contributions of dominant and less dominant prey categories. For each of these two data sets, a resemblance matrix was then constructed using the Bray Curtis resemblance measure. A two-way Permutational Analysis of Variance test (PERMANOVA; Anderson et al. 2008) was then conducted on each matrix to assess whether dietary composition differed significantly between species and/or size classes. Both factors were considered fixed and were crossed with each other, and the null hypothesis of no significant difference among groups was rejected if the significance level (P) was <0.05 . The components of variation value (COV) for each significant term was used to determine their relative importance.

Subsets of the above resemblance matrices were then subjected to one-way Analysis of Similarity tests (ANOSIM; Clarke and Green 1988) to further explore any significant differences in dietary composition detected by PERMANOVA. For both the %V and %W matrices, ANOSIM tests for size class differences were undertaken separately for each species, and vice versa. The criterion for rejecting the null hypothesis of no significant differences amongst groups was the same as that for PERMANOVA, and the extent of any significant differences were judged by the magnitude of the *R*-statistic.

To visualise any significant dietary composition differences between species and/or size classes, the distance among centroids was calculated for each species x size class combination and used to produce a resemblance matrix, which was then subjected to metric-MDS ordination. Confidence intervals (95%) of the averages for each species x size class combination were then calculated using the bootstrap averages routine (100 bootstraps per group), then plotted on a separate mMDS plot along with the group averages.

To determine which prey taxa were driving any significant differences in %V or %W among species and size classes, a shade plot (Clarke et al. 2014) was constructed from the complimentary dietary data. The pre-treated data was first averaged for each species x size class combination for all prey categories, then the data was used to construct a resemblance matrix defined between prey categories as Whittaker's index of association. This matrix was then subjected to a group-average hierarchical agglomerative cluster analysis along with a Similarity Profiles test (SIMPROF Type 3; Somerfield and Clarke 2013) to identify those points in the clustering procedure in which no significant

structure (i.e. differences in %V or %W) could be detected. The resulting dendrogram was used to seriate the order of prey categories along the y-axis of the shadeplot. Samples, displayed on the x-axis, were ordered by species then size class. Differences in the magnitude of average %V or %W values were visually depicted via monochromatic shading, with darker samples reflecting larger values.

2.4.2 Stable Isotope Analysis

Mean (\pm SD, standard deviation) values of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ signatures for each species and size class combination were plotted as a scatterplot (biplot) to illustrate any differences in their primary energy source ($\delta^{13}\text{C}$) and/or trophic level ($\delta^{15}\text{N}$). Inflated $\delta^{13}\text{C}$ values were considered to represent a more marine-derived diet, while lower values were considered to reflect dietary sources from freshwater/terrestrial origins (DeNiro and Epstein 1978, Burns et al. 1998).

A second biplot was constructed using the average (\pm SD) $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values for various fish and elasmobranch species and their potential prey recorded by Svensson et al. (2007) in the Walpole-Nornalup Estuary in April 2001. The sampling of prey taxa by Svensson et al. (2007) was conducted within the area of sparid overlap sampled in the current study. The average $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values for each sparid species in this study were also plotted on the same biplot of the Svensson et al. (2007) data to determine (i) whether sparid signatures from the current study were comparable to those recorded nearly two decades ago, and if so, (ii) which other taxa sampled by Svensson et al. (2007) are likely to be potential food sources or predators of the sparids sampled in this study.

3. Results

3.1 Overall characteristics of sparid gut contents

In total, 227 guts were examined across the three Sparidae species caught in the zone of spatial overlap during autumn 2018, 186 of which contained identifiable items and hence were included in subsequent dietary analyses. Identifiable items were found in 15-51 guts of each size class for each species, except for *C. auratus*, for which only five fish in smallest size class contained food. Only one large-sized *C. auratus* was caught within the area of sparid overlap but was found to have no identifiable prey items and was hence excluded from analyses. Further information on the proportion of full to empty guts, average estimated gut fullness and the most frequently preyed upon dietary taxa (%F) is provided for each sparid species and size class in Table 3.2. In terms of the gears used to capture sparids, seine nets were most effective for capturing *A. butcheri* and caught over 5 times more bream than rod & line fishing techniques (Table 3.1). Rod & line fishing and seine nets captured roughly equal numbers of *R. sarba*, whereas *C. auratus* were caught exclusively with rod & line techniques due to their close association with rocky substrates that would pose a snag risk to netting techniques (Table 3.1).

Table 3.1. Summary table of the gear type used to capture sparids in each size class. Values depict the total number of sparids caught with identifiable prey items within their entire gut contents.

	Size Class			Total
	0-150 mm	150-250 mm	>250 mm	
<i>Acanthopagrus butcheri</i>	20	51	28	99
Rod & Line		5	11	16
Seine Net	20	46	17	83
<i>Rhabdosargus sarba</i>	16	32	15	63
Rod & Line	3	18	6	27
Seine Net	13	14	9	36
<i>Chrysophrys auratus</i>	5	19		24
Rod & Line	5	19		24

3.1.1 *Acanthopagrus butcheri*

Food was present in the guts of 99 of the 109 *A. butcheri* that were caught, with 20, 51 and 28 individuals in the smallest, medium and largest size classes, respectively. The average estimated gut fullness across these 99 fish was 50% (SE=2.37), with the seagrass *Ruppia megacarpa* having the highest %F (51% of all guts), followed by the bivalve *Arthritica semen* (36%) and the red algae *Polisiphonia* spp. (32%; Table 3.2). Juveniles in the small size class preyed primarily on *A. semen* followed by *R. megacarpa* and Polychaeta spp., while individuals in the medium and large size classes targeted *R. megacarpa* and/or *Polisiphonia* spp. more frequently (Table 3.2).

3.1.2 *Rhabdosargus sarba*

Of the 73 *R. sarba* examined, 63 had identifiable prey items with each size class consisting of 16 small, 32 medium and 15 large-sized individuals. While the average estimated gut fullness was lower than that for *A. butcheri* (32%; SE=2.78), *R. sarba* most commonly preyed upon the same three dietary categories as *A. butcheri*, and in very similar proportions (Table 3.2). However, fish in the smallest size class fed more frequently on amphipods such as *P. excavatum* than the other two sparids (%F=67%), whilst *R. megacarpa* consumption increased progressively throughout maturity (75% for the largest size class; Table 3.2).

3.1.3 *Chrysophrys auratus*

Due to difficulties in capture (i.e. limited captures in gill and seine nets), only five small *C. auratus* were found with food items in their gut, and due to the scarcity of larger-sized individuals within the Walpole-Nornalup Estuary, the medium and large size classes of this species consisted of 19 and zero individuals, respectively. *Chrysophrys auratus* also had the highest proportion of empty guts, with only 24 of the 46-fish caught having identifiable prey items within their stomach and intestines. The average estimated gut fullness for these 24 fish was 35% (SE=3.81). The consumption of different prey taxa was much more evenly spread for *C. auratus* in comparison to the other two sparids, with the bivalve, *Xenostrobus securis* having the highest %F value at only 24%. Whilst the small *C. auratus* targeted tanaids, barnacles (Cirripedia spp.), *X. securis* and *P. excavatum* with equal frequency, their diet became more varied with increasing size, with *X. securis* and decapods being the largest contributors in the medium size class (Table 3.2).

Table 3.2. Percentage frequency contribution (%F) of each dietary category for each size class (S, M and L) in which “Total” refers to the %F value across all size classes. Dominant dietary categories (%F>25%) are shaded in grey. Note that dietary categories in the “Other” grouping have not been included in subsequent analyses, but are shown here to indicate the relative contributions of identifiable vs unidentified gut contents.

Taxa	Dietary Categories	<i>Acanthopagrus butcheri</i>				<i>Rhabdosargus sarba</i>				<i>Chrysophrys auratus</i>		
		Total	S	M	L	Total	S	M	L	Total	S	M
Seagrass	<i>Ruppia megacarpa</i>	51	43	61	40	52	17	59	75	4	0	5
Chlorophyta	<i>Rhizoclonium implexum</i>	2	0	2	3	0	0	0	0	0	0	0
Rhodophyta	Rhodophyta spp.	1	0	0	3	0	0	0	0	4	0	5
	Polysiphonia spp.	32	17	36	37	25	11	33	19	7	0	8
Polychaete	Nereididae spp.	3	4	4	0	0	0	0	0	2	14	0
	Polychaeta spp.	29	43	32	13	11	6	8	25	2	0	3
Crustacea	Tanaidacea spp.	6	0	7	10	5	6	5	6	13	29	11
	Cirripedia spp.	2	0	0	7	0	0	0	0	11	29	8
	<i>Paracorophium excavatum</i>	12	30	7	7	25	67	15	0	4	29	0
	Amphipoda spp.	7	13	9	0	14	28	13	0	2	14	0
	Isopoda spp.	1	4	0	0	0	0	0	0	0	0	0
	Aoridae spp.	1	0	2	0	0	0	0	0	0	0	0
	Decapoda spp.	3	0	4	3	3	0	5	0	11	0	13
	<i>Amarinus laevis</i>	1	0	2	0	0	0	0	0	4	0	5
	Crustacea spp.	7	17	5	3	7	11	5	6	2	0	3
Bivalve	<i>Xenostrobus securis</i>	13	9	4	33	14	11	18	6	24	29	24
	<i>Arthritica semen</i>	36	65	30	23	27	33	23	31	7	0	8
	<i>Tellina deltoidalis</i>	4	4	5	0	3	0	3	6	0	0	0
	Bivalvia spp.	8	4	9	10	4	0	3	13	9	14	8
	<i>Arcuatula senhousia</i>	9	13	11	3	8	17	0	19	2	14	0
	<i>Hydrococcus brazieri</i>	0	0	0	0	0	0	0	0	2	0	3
Insects	Chironomid larvae	1	4	0	0	3	0	0	13	2	0	3
	<i>Heteronychus arator</i>	1	0	0	3	0	0	0	0	0	0	0
Teleost	<i>Leptatherina presbyteroides</i>	4	0	2	10	7	0	3	25	4	0	5
	Teleostei spp.	10	9	13	7	5	0	3	19	9	0	11
	Atherinidae spp.	4	0	5	3	0	0	0	0	4	0	5
Other	Digested Material	37	13	39	50	25	28	28	13	11	14	11
	Sand	6	17	2	7	7	6	8	6	0	0	0

3.2 Differences in gut content composition among sparid species and size classes

Dietary composition, based on both the volumetric (%V) and weight (%W) contributions of all prey categories to fish gut contents, differed significantly among sparid species, size classes and the interaction between these main effects ($P = 0.001$; Table 3.3). For both data types, all of the above model terms were of similar importance (i.e. had similar COV values), but with size class being slightly more influential for %V and the interaction being most important for %W.

Table 3.3. Mean squares (MS), Pseudo-F values (F), significance levels (P) and Components of Variation values (COV) for a two-way PERMANOVA test of dietary composition (based on both %V and %W gut content data) amongst the three Sparidae species (*Acanthopagrus butcheri*, *Rhabdosargus sarba* and *Chrysophrys auratus*) and size classes (0-150, 150-250 and >250 mm). Degrees of freedom, $d.f.$

	Effects	$d.f.$	MS	F	P	COV
%V	Species	2	26231	4.975	0.001	15.402
	Size Class	2	31448	5.964	0.001	17.391
	Species x Size Class	3	22377	2.829	0.001	15.836
	Residuals	178	4.69E-05			51.345
%W	Species	2	22390	4.621	0.001	14.093
	Size Class	2	28394	5.405	0.001	16.756
	Species x Size Class	3	25342	3.216	0.001	17.549
	Residuals	180	4.73E-05			51.251

Given the significant interaction effect for both %V and %W, further examination of size class differences was undertaken using separate one-way ANOSIM tests for each species (Table 3.4), and species differences were similarly explored separately for each size class (Table 3.5).

Significant dietary differences were detected among size classes for both *A. butcheri* and *R. sarba*, but not in the case of *C. auratus* for both the %V and %W data sets (Table 3.4). The overall extent of these significant differences was, however, small (i.e. Global $R=0.138-0.293$). Dietary differences were greatest between the smallest and largest size classes for both *A. butcheri* and *R. sarba* but were far more distinct for the latter species ($R=0.539$ for %V and 0.581 for %W vs $0.159-0.184$ for *A. butcheri*; Table 3.4). Significant and moderate differences were also found between small and medium-sized *R. sarba*, while no significant dietary differences were detected between medium and larger-sized *R. sarba* or between small and medium *A. butcheri* based on %V data (Table 3.4).

Table 3.4. *R*-statistic and/or *P* values for global and pairwise comparisons from one-way ANOSIM tests of size class differences in dietary composition (small, S: 0-150 mm, medium, M: 150-250 mm, large, L: >250 mm) for each of the three *Sparidae* species, based on both %*V* and %*W* gut content data. Significant pairwise tests are in bold. Note that the test for *C. auratus* only includes fish from the small and medium size classes.

		<i>Acanthopagrus butcheri</i>			<i>Rhabdosargus sarba</i>			<i>Chrysophrys auratus</i>		
%V		Global <i>P</i> = 0.001			Global <i>P</i> = 0.001			Global <i>P</i> = 0.183		
		Global <i>R</i> = 0.138			Global <i>R</i> = 0.280			Global <i>R</i> = 0.092		
		S	M	L	S	M	L			
		S	0.094	0.159		0.346	0.539			
%W		M		0.150			0.082			
		Global <i>P</i> = 0.001			Global <i>P</i> = 0.001			Global <i>P</i> = 0.532		
		Global <i>R</i> = 0.158			Global <i>R</i> = 0.293			Global <i>R</i> = -0.016		
		S	M	L	S	M	L			
		S	0.133	0.184		0.411	0.581			
		M		0.156			0.031			

Species differences in dietary composition were significant for both the small and medium size classes, but not the largest one (Table 3.5). However, the extent of these significant differences was again relatively small (Global *R*=0.222-0.288 for %*V* and 0.22-0.296 for %*W*; Table 3.5). The greatest species differences occurred in the smallest size class, and specifically between *A. butcheri* and *C. auratus* (%*V*: *R* = 0.572; %*W*: *R* = 0.553). In the medium size class, moderate differences were detected between *C. auratus* and both *A. butcheri* and *R. sarba* (%*V*: *R* = 0.425-0.478; %*W*: *R* = 0.369-0.467), but *A. butcheri* and *R. sarba* diets were not significantly different based on either data set (Table 3.5).

Table 3.5. *R*-statistics and *P* values for global and pairwise comparisons from one-way ANOSIM tests of species differences in the dietary composition (AB: *Acanthopagrus butcheri*, RS: *Rhabdosargus sarba*, CA: *Chrysophrys auratus*) for each size class, based on both %V and %W gut content data. Significant pairwise tests are in bold.

		Size Class					
		Small		Medium		Large	
%V		Global <i>P</i> = 0.001		Global <i>P</i> = 0.001		Global <i>P</i> = 0.074	
		Global <i>R</i> = 0.288		Global <i>R</i> = 0.222		Global <i>R</i> = 0.079	
		AB	RS	CA	AB	RS	CA
		AB	0.223	0.572		-0.001	0.478
%W		RS		0.264			0.425
		Global <i>P</i> = 0.001		Global <i>P</i> = 0.001		Global <i>P</i> = 0.065	
		Global <i>R</i> = 0.296		Global <i>R</i> = 0.220		Global <i>R</i> = 0.087	
		AB	RS	CA	AB	RS	CA
		AB	0.252	0.553		0.000	0.467
		RS		0.228			0.369

The extent of the dietary composition differences among species and size classes were illustrated by the centroid mMDS ordination plots shown in Figure 3.1 (%V data) and Figure 3.3 (%W data). Note that since the patterns in these two plots were very similar, the following provides a general description of both.

The main drivers for the species x size class interaction detected by PERMANOVA were clearly the notably larger difference (longer trajectory) between small and medium *R. sarba* than for any other species x size class combination, as well as the comparatively smaller differences between medium and large *R. sarba* (Figs 3.1 and 3.3). With respect to overall species differences, both small and medium-sized *C. auratus* were clearly separated from all size classes of *A. butcheri* and *R. sarba*,

illustrating the comparatively large differences in the dietary composition of former species. Centroids of the smallest size category of all species were also more obviously separated from those for the medium and larger size classes, which were intermingled towards the bottom left of the plots (Figs 3.1 and 3.3).

Figures 3.2 and 3.4, derived from the %V and %W data, respectively, reflect the variation around each of the species x size class group averages by depicting the areas (ellipses) in which 95% of the bootstrapped averages are expected to fall. For both plots, the highest variation was found for small *C. auratus*, most likely reflecting the smaller sample size for this group (see subsection 3.1.3). The medium size classes for each species had the smallest amount of variation as the sparids were most commonly caught at these lengths and hence had a larger sample size than smaller or larger sized individuals (Figs 3.2 and 3.4).

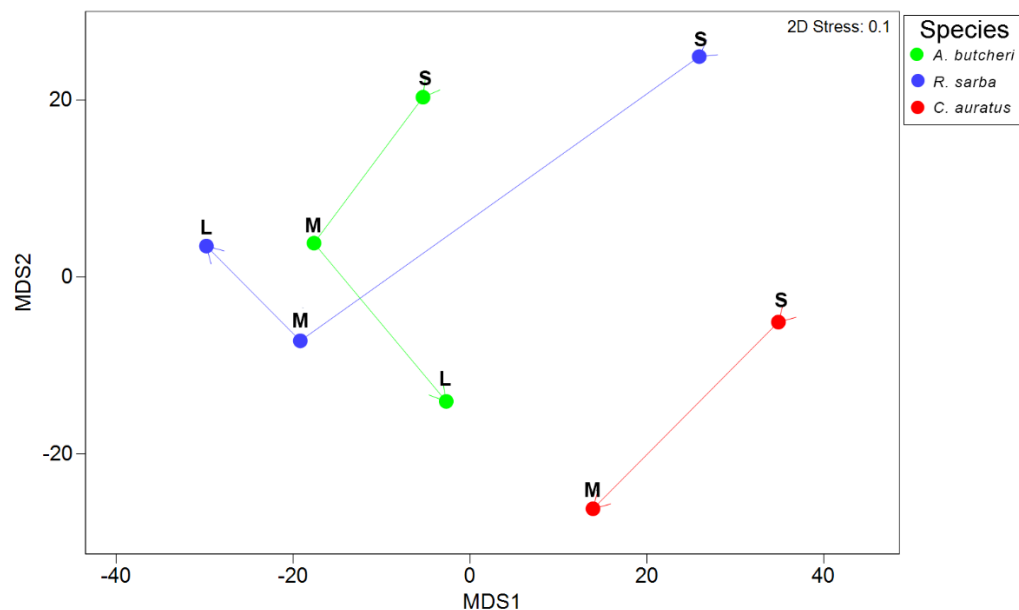


Figure 3.1. mMDS centroid ordination plot derived from the %V dietary composition data for each species and size class combination. Sample labels reflect fish size class (S, small; M, medium; L, large).

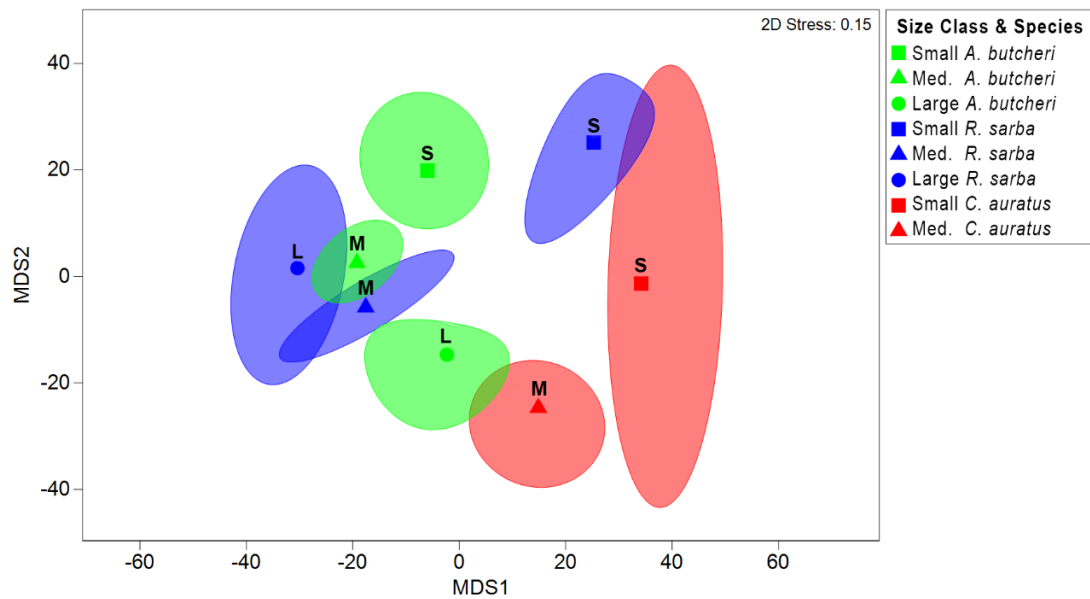


Figure 3.2. mMDS ordination plot constructed from the group and bootstrapped averages of %V dietary data for each species and size class combination (S, small; M, medium; L, large). Ellipses around each group average represent the 95% confidence boundaries from 100 bootstrap replicates.

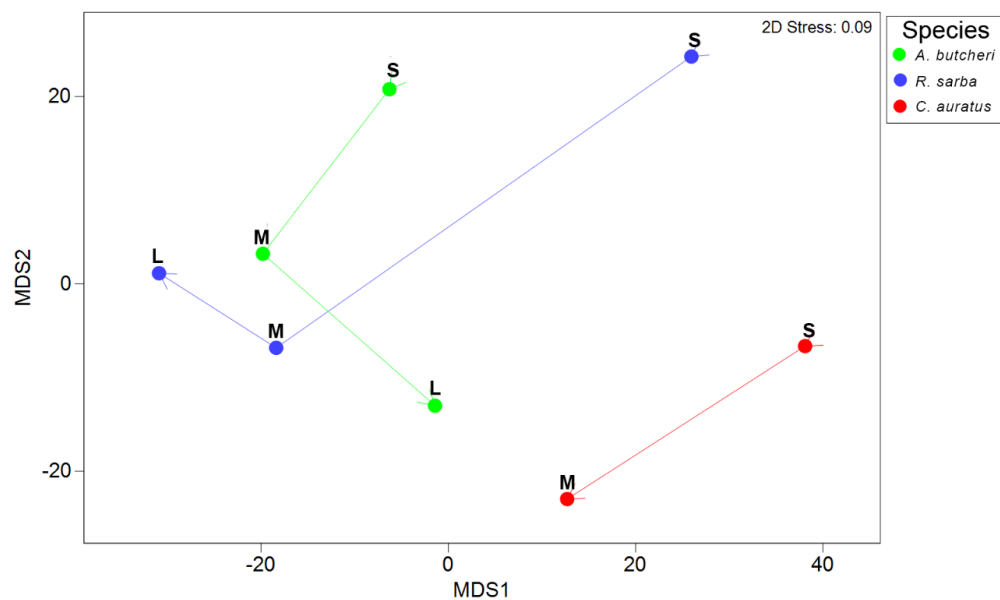


Figure 3.3. mMDS centroid ordination plot derived from the %W dietary composition data for each species and size class combination. Sample labels reflect fish size class (S, small; M, medium; L, large).

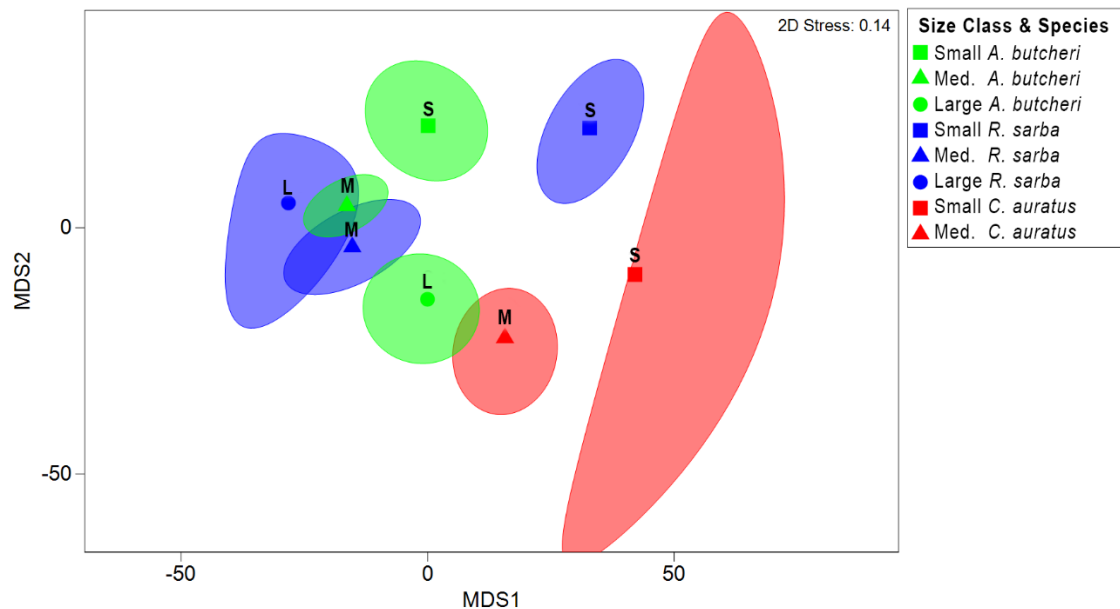


Figure 3.4. mMDS centroid ordination plot constructed from the bootstrapped averages of %W dietary data for each species and size class (S, small; M, medium; L, large) combination. Ellipses around each group centroid represent the 95% confidence boundaries from 100 bootstrap replicates.

The shadeplots shown in Figs 3.5 and 3.6, derived from %V and %W data, respectively, illustrate the key dietary categories most responsible for driving the observed differences among sparid species and size classes. Overall, the trends in these two shade plots were very similar. The following thus provides a general description of both plots, with any differences highlighted where relevant.

The species x size interaction, which as outlined above, mainly reflected the much greater dietary differences between small and medium *R. sarba*. Small *R. sarba* fed mainly on *P. excavatum* and other amphipods, whereas medium-sized individuals were much more herbivorous, feeding predominately on *R. megacarpa* and *Polisiphonia* sp. (Figs 3.5 and 3.6).

Chrysophrys auratus consumed a much more carnivorous diet compared to the other two sparids and very rarely targeted seagrass or any algal species (Figs 3.5 and 3.6).

Whilst small-sized *C. auratus* overlapped with the other sparids in terms of amphipod consumption, medium-sized individuals consumed much more *X. securis*, Decapoda spp. and Tanaids than the other sparids of equivalent size. *Acanthopagrus butcheri* fed primarily on *R. megacarpa*, *A. semen* and small burrowing amphipod species such as *P. excavatum* (Figs 3.5 and 3.6). The shadeplots illustrated the moderate significant difference in diet between small and large *A. butcheri*, with smaller-sized individuals targeting amphipods (i.e. *P. excavatum*) and *R. megacarpa* and larger individuals consuming a broader omnivorous diet containing larger volumes of *X. securis*, atherinids such as *Leptatherina presbyteroides*, *R. megacarpa* and *Polisiphonia* spp. (Figs 3.5 and 3.6). Overall, the strongest amount of resource overlap was observed between *A. butcheri* and *R. sarba*, that both consumed large quantities of *R. megacarpa* in all three size classes (Figs 3.5 and 3.6).

While the trends in the shadeplots constructed from %V and %W data were similar, some differences were apparent. For example, ANOSIM detected a significant difference in dietary composition between small and medium-sized *A. butcheri* based on %W, but not %V (Table 3.4). This significant difference mainly reflected a greater %W of *R. megacarpa* in the medium fish while the opposite was true for *A. semen* and *P. excavatum* in the small fish (Figure 3.6). Furthermore, the diets of small *R. sarba* and *C. auratus* were significantly different based on the %V data but not %W (Table 3.5). This is likely due to the greater proportions of *Arcuatula senhousia* (Asian bag mussels) and *Nereidae* spp. (Polychaetes) consumed by small *C. auratus* in terms of the %V (Figure 3.5).

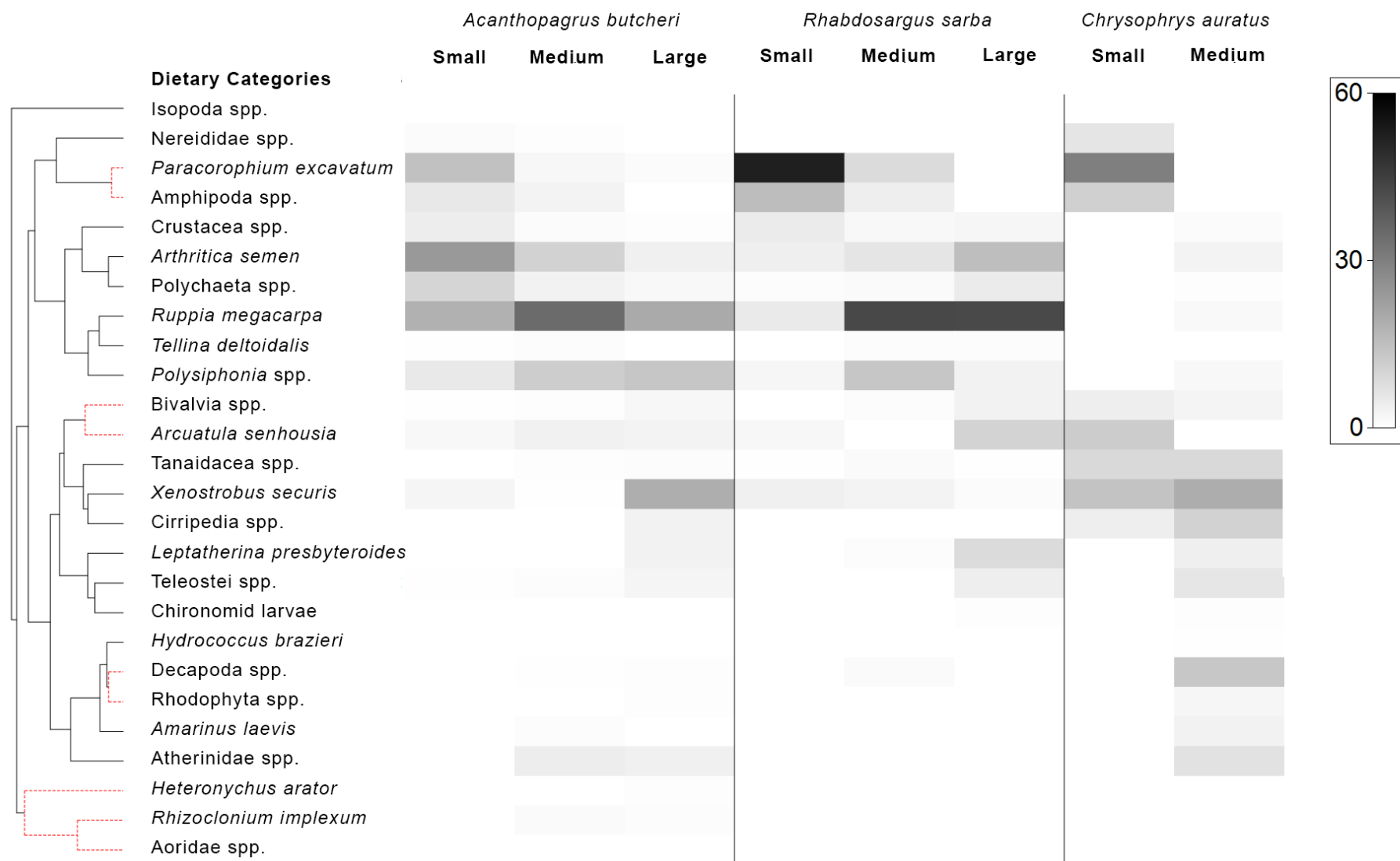


Figure 3.5. Shade plot of the pre-treated average %V contribution of the most prevalent prey categories consumed by each species and size class x (Small: 0-150, Medium: 150-250 and Large: >250 mm). Prey categories were ordered by a hierarchical cluster analysis of their mutual associations across species size class groups. Dashed red lines in the dendrogram indicate prey categories with significantly similar patterns in %V, as detected by SIMPROF.

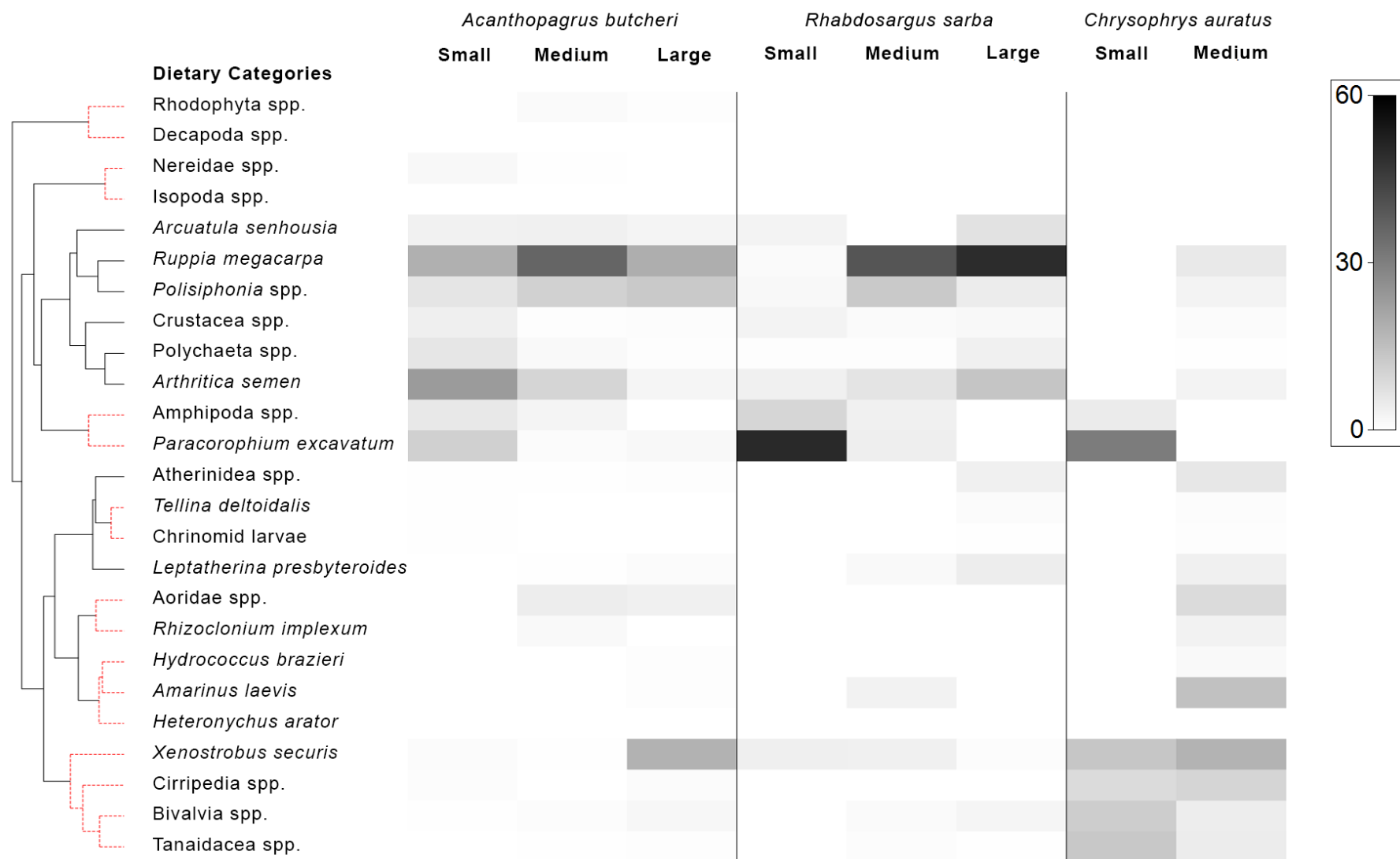


Figure 3.6. Shade plot of the pre-treated average %W contribution of the most prevalent prey categories consumed by each species and size class (Small: 0-150, Medium: 150-250 and Large: >250 mm). Prey categories were ordered by a hierarchical cluster analysis of their mutual associations across species x size class groups. Dashed red lines in the dendrogram indicate prey categories with significantly similar patterns in %W, as detected by SIMPROF.

3.3 Isotopic composition of each sparid species and size class

The average (\pm SD) $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ signatures from muscle samples of fish from each species and size class were plotted to examine any differences in their trophic level ($\delta^{15}\text{N}$) and/or the extent of marine vs terrestrial dietary influences ($\delta^{13}\text{C}$). The resultant biplot showed gradational trends among both species and their size classes, but the pattern and extent of differences varied in each case (Figure 3.7). These differences are explored in more detail for both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ below.

3.3.1 Trends in average $\delta^{13}\text{C}$ among species and size classes

Mean $\delta^{13}\text{C}$ values differed most obviously between *A. butcheri* and *C. auratus*, with the lower values for the former species indicating a more freshwater/terrestrial-derived diet and higher values for the latter reflecting a more marine-derived diet (Figure 3.7). In particular, the smallest size class of *A. butcheri* had the lowest $\delta^{13}\text{C}$ values and their diet became progressively more marine-influenced as they matured, with the mean signature for the largest *A. butcheri* being only slightly less than that for *C. auratus*. Average $\delta^{13}\text{C}$ signatures for all size classes of *R. sarba* indicated a brackish/slightly marine derived diet, but unlike the situation for *A. butcheri*, the largest size class fed on a slightly more freshwater/terrestrial-derived diet than the smaller size classes (Figure 3.7).

Despite the above species and size differences in mean $\delta^{13}\text{C}$ values, there was considerable overlap in their corresponding standard deviation values (Figure 3.7). Larger *R. sarba* as well as medium to large *A. butcheri* had the highest amount of variability, suggesting their diet was more varied and opportunistic. Conversely, smaller *C. auratus*

and *R. sarba* had the smallest variability about their mean $\delta^{13}\text{C}$ values, suggesting a more specialised diet (Figure 3.7).

3.3.2 Trends in average $\delta^{15}\text{N}$ among sparid species and size classes

Mean $\delta^{15}\text{N}$ values showed that *A. butcheri*, and particularly small and medium-sized fish, occupied a higher trophic level than all other species and size classes, especially compared to small *R. sarba* and medium-sized *C. auratus* (Figure 3.7). Additionally, the trophic level decreased with increasing size in *A. butcheri* and to a lesser extent in *C. auratus*, but increased with size in *R. sarba*.

As for the mean $\delta^{13}\text{C}$ values, the standard deviations around mean $\delta^{15}\text{N}$ values were considerable in some cases and often overlapped with others, especially at lower trophic levels (bottom right of the biplot). The greatest variation around mean $\delta^{15}\text{N}$, however, was detected for large and medium-sized *R. sarba* (Figure 3.7).

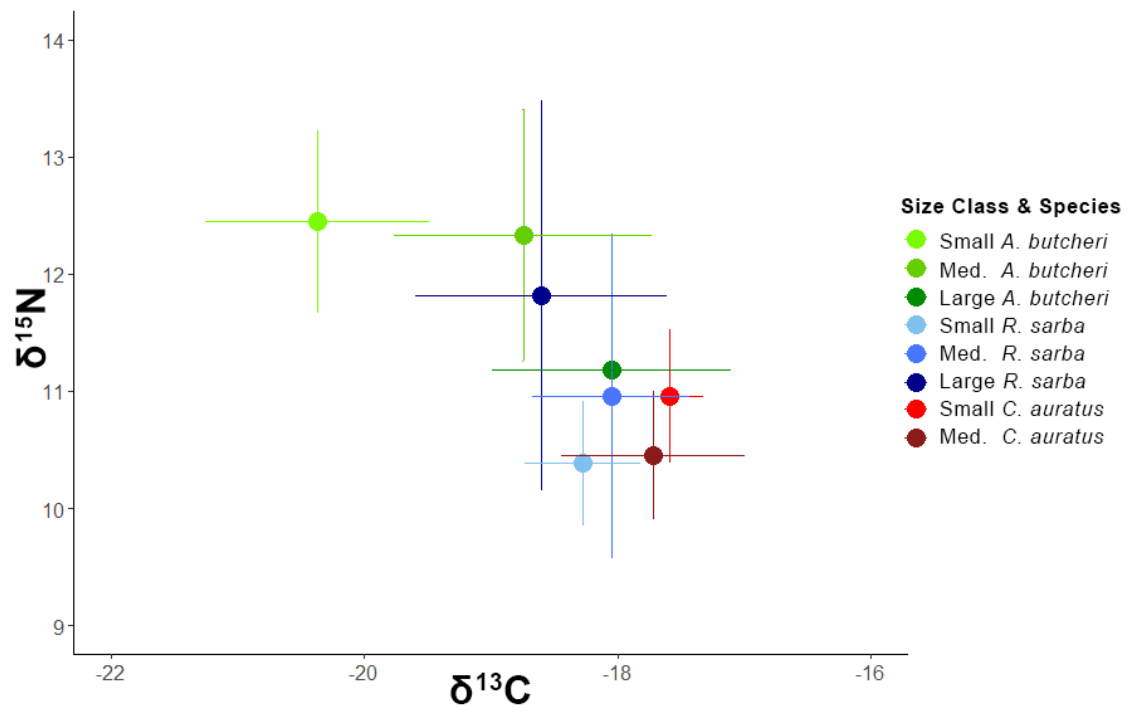


Figure 3.7. Biplot of the mean (\pm SD) $\delta^{13}\text{C}$ vs $\delta^{15}\text{N}$ values of the three sparid species and size classes.

3.4 Isotopic signatures of sparid species in relation to those of potential predators and prey

Comparison of the trophic levels and primary energy sources of the three sparids recorded in this study with the single sparid species sampled by Svensson et al. (2007) in 2001 (*A. butcheri*) showed that they generally occupied a similar area on the biplot and had a high degree of isotopic overlap (Figure 3.8). However, the $\delta^{13}\text{C}$ values for *A. butcheri* recorded by Svensson et al. (2007) were higher (more marine-influenced) than those recorded for this species in the study, and comparison of the corresponding $\delta^{15}\text{N}$ values indicated *A. butcheri* were feeding at a lower trophic level in 2001 than 2018.

Overall, the $\delta^{13}\text{C}$ signatures for the sparids from both this study and that by Svensson et al. (2007) were lower than those for any other fish species sampled,

particularly compared to Trevally (*Pseudocaranx* sp.). All of the elasmobranch species sampled (Southern eagle ray *Myliobatis tenuicaudatus*, Western Shovelnose Ray *Aptychotrema vincentiana* and Gummy shark *Mustelus antarcticus*) had even higher average $\delta^{13}\text{C}$ values, reflecting their more marine dietary sources (Figure 3.8). The isotopic values of the sparids did show some overlap, however, with the Common sand crab (*Ovalipes australiensis*) and also other teleost species such as the Bridled goby (*Arenigobius bifrenatus*) and Estuary cobbler (*Cnidogobius microcephalus*; Figure 3.8).

Potential prey of the sparids sampled in the current study (i.e. taxa positioned beneath these samples on the biplot) included teleosts such as *Leptatherina presbyteroides*, the Blue swimmer crab (*Portunus armatus*), gastropods (*Nassarius* sp.), bivalves (*Katelysia* spp. and *Tellina* spp.) and algae (*Chaetomorpha linum*; Figure 3.8). Whilst some of these prey items were identified as dietary categories in the gut content analyses undertaken in the current study (namely *L. presbyteroides* and *Tellina* spp.), the remainder were not recorded in any of the sparid guts examined (Table 3.2).

Australian herring (*Arripis georgianus*) and Gummy shark (*M. antarcticus*) had the highest mean $\delta^{15}\text{N}$ values recorded by Svensson et al. 2007, and fed at a higher trophic level than the three sparid species. The positioning of *A. georgianus* above the sparid species on the biplot indicates it may be potential predators of these species (Figure 3.8).

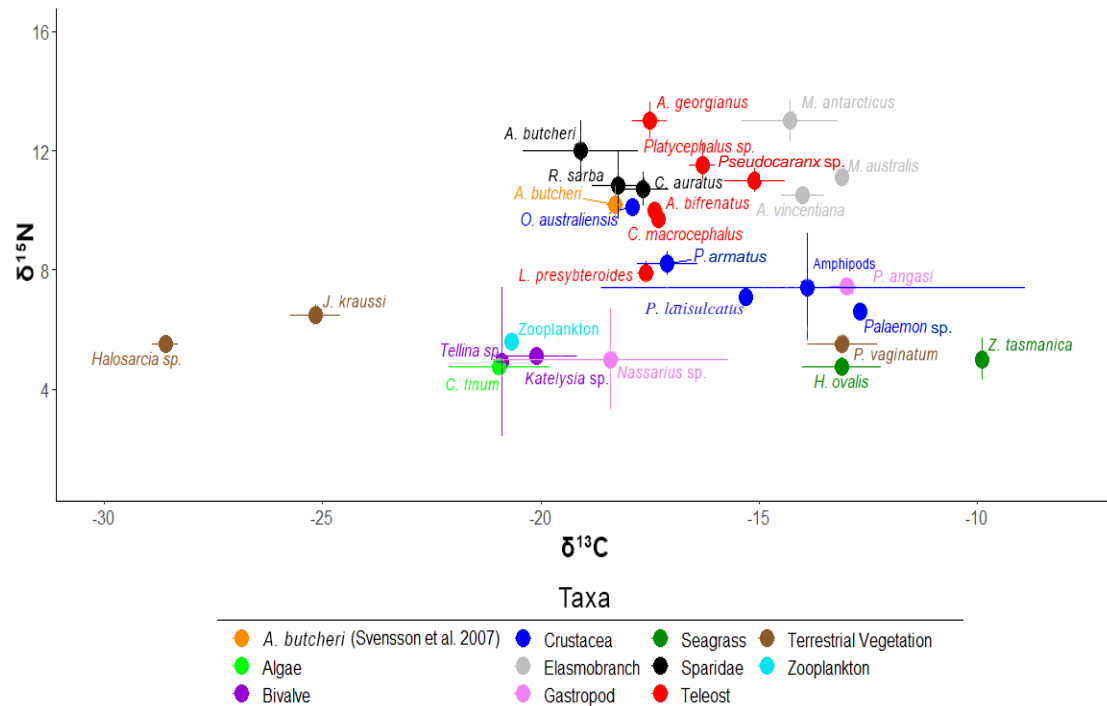


Figure 3.8. Biplot of the mean (\pm SD) $\delta^{13}\text{C}$ vs $\delta^{15}\text{N}$ isotopic signatures from the three sparids collected in this study (denoted by black symbols) and from other biota collected in the Walpole-Nornalup Estuary in 2001 by (Svensson et al. 2007). Species consisted of: *Acanthopagrus butcheri*, *Aptychotrema vincentiana*, *Arenigobius bifrenatus*, *Arripis Georgianus*, *Chaetomorpha linum*, *Chrysophrys auratus*, *Cnidoglanis microcephalus*, *Halophila ovalis*, *Juncus kraussi*, *Leptatherina presbyteroides*, *Mustelus antarcticus*, *Myliobatis australis*, *Ovalipes australiensis*, *Panaeus latisulcatus*, *Paspalum vaginatum*, *Philine angasi*, *Portunus armatus*, *Rhabdosargus sarba* and *Zostera tasmanica*.

4. Discussion

Whilst the degree of dietary overlap differed to varying extents across species and size classes, the greatest amount of overlap was observed between *A. butcheri* and *R. sarba* which both fed on similar proportions of algae and seagrass after reaching a TL of 250 mm. In terms of trophic partitioning, small to medium sized *A. butcheri* fed at the highest trophic level and had a notably higher freshwater dietary influence compared to the other sparids. Both dietary assessment techniques highlighted different aspects of estuarine fish

diets, with SIA providing an insight into the trophic partitioning and long-term feeding trends (freshwater vs marine). Gut content analysis on the other hand, facilitated the quantitative assessment of species and size class differences, and provided information on the actual prey sources responsible for the observed dietary differences. With the recent and ongoing impacts of climate change towards both, estuarine morphology and water chemistry, the abundance of marine species such as *C. auratus* and *R. sarba* will likely increase in estuarine environments like the Walpole-Nornalup Estuary, creating further competition for resources amongst estuarine and marine fishes. Combined with the intense recreational fishing pressures in the south-west region, this could lead to substantial declines in the growth rates and productivity of these key-fishery species and in particular, the highly targeted *A. butcheri*. The information derived from this study will contribute to the understanding of estuarine and marine fish resource partitioning and the degree of resource competition amongst cofamilial fish species. Furthermore, this study has provided important baseline information regarding the essential food sources required by these key fishery species, allowing environmental management authorities to understand what regions and habitat types should be protected to best preserve the fishing amenity and ecosystem health of the region.

4.1 Differences in gut content composition among *Sparidae* species

The largest differences in gut content composition amongst species was observed between *A. butcheri* and *C. auratus*. The former species primarily consumed seagrass (*R. megacarpa*), algae (*Polisiphonia* spp.) and bivalves (*A. semen* at earlier life stages and *X. securis* at later stages), whereas the latter consumed a more varied suite of invertebrate

and fish prey (e.g. tanaids, *X. securis*, Cirripedia spp., *P. excavatum* and decapods) and, unlike both other sparids, rarely targeted seagrass or algae. The trends observed in *A. butcheri* diet in the current study correspond with the findings of Sarre and Potter (1999) in the Walpole-Nornalup from 1995/96, in which the most frequently consumed dietary categories were *R. megacarpa* and various filamentous algal species.

Differences in the structural habitat preferences of *A. butcheri* and *C. auratus* within the area of overlap in the Walpole-Nornalup Estuary may possibly contribute to these observed differences in diet. During the current study, *A. butcheri* was more commonly encountered when line-fishing around wooden snags, whereas *C. auratus* was most frequently taken near submerged rocky habitat (pers. obs.). Other studies have also reported *A. butcheri* to be closely associated with submerged woody debris such as sticks and logs (Hindell 2007, Hindell et al. 2008), and *C. auratus* with rocky substrates (Hartill et al. 2003, Harasti et al. 2015, Fowler et al. 2017). In the Walpole-Nornalup Estuary, submerged wooden structures provide an ideal substrate for algal attachment whereas rocky substrates support both algae and a range of invertebrates such as crustaceans, barnacles and filter feeding bivalves (Hindell et al. 2008, Fowler et al. 2017). The apparent habitat preferences of these species therefore expose them to different suites of potential prey species, as reflected in their dietary composition.

Acanthopagrus butcheri and *R. sarba* both shared similar omnivorous diets with dietary overlap particularly occurring in the medium-large size classes. *Ruppia megacarpa*, *Polisiphonia* spp. and *A. semen* were consumed at very similar frequencies and in similar volumes and/or weights indicating that considerable food resource overlap occurs between these two sparids species. Similarly, *R. sarba* has been found to become

progressively herbivorous by Peng (2003) in the Swan-Canning Estuary, who also proposed that resource overlap and partitioning would likely occur between these two species when co-occurring in estuarine systems in south-western Australia.

However, whilst resource overlap occurred between *A. butcheri* and *R. sarba*, significant but small dietary differences were apparent between the smallest size classes of these species, with *A. butcheri* consuming substantially more seagrass and *A. semen*, and *R. sarba* consuming considerably more amphipods, particularly *P. excavatum*. Moreover, it is possible that resource overlap between these species may be minimised at different times of the year throughout this estuary, particularly as both species are known to be highly opportunistic feeders (Blaber 1984, Sarre et al. 2000, Peng 2003, Chuwen et al. 2007, Linke 2011). From the acoustic telemetry work by Yeoh (2018), it was found that *R. sarba* and *C. auratus* were displaced further downstream from the Frankland River during winter when freshwater flows occurred. Consequently, it is likely that resource competition would be reduced during the wetter seasons, and strongest during summer and autumn when higher salinities allow the two marine-estuarine opportunist species to move upstream, increasing their overlap with *A. butcheri* (Yeoh 2018).

Acanthopagrus butcheri and *R. sarba* had limited instances of empty guts, with >85% of individuals containing at least one identifiable prey item. *Chrysophrys auratus* however, had a large amount of empty guts, with only 52% of guts found to contain prey items. A very similar trend was observed in *C. auratus* caught in shallow marine waters along the south-west coast of Australia by French et al. (2012), of which only 52% of individuals under 400 mm in length had items in their guts. This pattern likely reflects the predatory, carnivorous feeding habits of this species, which typically feeds on smaller

quantities of protein-rich prey that are less readily available than for instance, seagrass and algae (Woodward and Hildrew 2002).

Overall, the dietary trends observed between the three Sparidae species supported the first hypothesis posed in this study, that the greatest dietary overlap would occur between *A. butcheri* and *R. sarba* due to their omnivorous feeding strategies, and that *C. auratus* would have a more carnivorous diet.

4.2 Differences in gut content composition among size classes

In general, the greatest gut content differences were found between the small and large size classes, although this was not the case for *C. auratus* in which no significant size class differences were detected, and no individuals in the large size category with food in their gut were captured. This hence provided evidence against the original hypothesis in that the greatest differences in dietary composition would occur in the largest size class as the adults would have more specialised diets due to developments in morphology and habitat preferences (Marais 1980, Lukoschek and McCormick 2001, Svanbäck and Bolnick 2007). This was unexpected as typically juvenile fish of different species share similar diets as the smaller mouth gape and rudimentary dentition limits the range of possible prey items able to be captured and consumed (Stoner and Livingston 1984, Wainwright and Richard 1995, Piet 1998, Juanes et al. 2002). Perhaps the lack of significant differences observed in larger-sized individuals (i.e. *A. butcheri* and *R. sarba*) was because these species became increasingly herbivorous with ontogeny and had strong overlap in terms of seagrass and algal consumption. This resource overlap was likely avoided by the juvenile sparids as whilst they primarily fed on the same prey sources

(namely, *P. excavatum* and other amphipods), they did so in very different proportions which would hence contribute to the significant differences between them in terms of both the %V and %W.

Pronounced ontogenetic shifts were identified between the smallest and largest size classes of both *A. butcheri* and *R. sarba* but were absent between the two size classes of *C. auratus* examined. The juveniles of both former species fed primarily on amphipods, including *P. excavatum*, as well as *A. semen* in the case of juvenile *A. butcheri*. Such differences in juvenile diets presumably help these two confamilial species to reduce inter-specific competition for resources during early life stages. With increasing size, both *A. butcheri* and *R. sarba* consumed more plant material (*Polisiphonia* spp, and/or *R. megacarpa*) and the first of these species also ate considerable volumes of the larger bivalve *X. securis*. As a result, the degree of dietary overlap, and presumably also resource competition between these species increased with increasing size (Chuwen et al. 2007).

Similar findings were reported in the Walpole-Nornalup Estuary by Sarre et al. (2000), in which smaller *A. butcheri* (>200 mm) primarily targeted polychaetes and decapods whereas larger fish consumed far greater amounts of macrophytes such as *Juncus* sp. and *R. megacarpa*. Similar ontogenetic shifts were also identified for *A. butcheri* in the Wellstead Estuary (Sarre et al. 2000), and for *R. sarba* in the Swan-Canning Estuary (Peng 2003), both of which became more herbivorous with maturity/increasing size.

It is possible that the greater quantities of energy-rich prey consumed by juvenile *A. butcheri* and *R. sarba* enabled them to grow more quickly to a size at which they were less vulnerable to predation. In contrast, larger fish would have less pressure for rapid

growth, and their tendency to target readily-available macrophytes would also reduce intra-specific competition with their juvenile counterparts.

The lack of significant dietary shifts between the two size classes of *C. auratus* examined in this study concurs with the findings of other researchers. For example, French et al. (2012) and Usmar (2012) found that juvenile snapper <100 mm in size primarily fed on amphipods and ophiuroid echinoderms in coastal waters, then switched to feeding on larger teleosts, brachyuran crabs and bivalves above ~300 mm in size. Given the typical absence of *C. auratus* at these larger size classes within the estuary (Yeoh 2018), it is likely that any ontogenetic shifts in diet of this species would occur only after the emigration to the marine environment.

4.3 Primary carbon sources and trophic positioning of Sparidae species

Both *C. auratus* and *R. sarba* were found to have similar $\delta^{13}\text{C}$ signatures that indicated a brackish/slightly marine-derived diet, whereas *A. butcheri* had a more freshwater/terrestrial sourced diet, particularly among small to medium-sized fish. These findings supported part of the third hypothesis posed in this study, namely that *C. auratus* would have a more marine-influenced diet than *A. butcheri*. However, contrary to this hypothesis, substantial overlap occurred between the $\delta^{13}\text{C}$ signatures of small to medium *R. sarba* and *C. auratus*. It was further hypothesised in this study that *C. auratus* would have the highest trophic positioning of the three sparids examined, however the findings showed that *A. butcheri*, and particularly its small to medium size classes, had a higher trophic level than both *R. sarba* and *C. auratus*.

Larger-sized *R. sarba* as well as *A. butcheri* from all size classes had the highest amount of variability around their mean $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values, which suggests their diet was more varied and likely more opportunistic than the other sparids (Figure 3.7). This coincides with the findings from other sparid dietary studies, as these two species in particular are well-known opportunistic feeders (Blaber 1984, Sarre et al. 2000, Peng 2003, Chuwen et al. 2007, Linke 2011).

The notably higher trophic level of small to medium *A. butcheri* compared with *C. auratus* was unexpected, given the largely carnivorous diet of the latter species including higher order prey such as crustaceans, decapods and fish, as found in this and other studies (Colman 1972, Peng 2003, French et al. 2012, Usmar 2012). The contradictory findings between GCA and SIA could possibly reflect the difference between methods in the time scale over which diet is measured, and the snapshot nature of the current study. It is possible that with further resampling across multiple seasons, medium and larger-sized *A. butcheri* may be found to consume greater quantities of teleosts and higher-order prey than was apparent from this study.

Smaller-sized *A. butcheri* fed at notably higher trophic levels than their adult counterparts, whereas the opposite was true for *R. sarba*. While it is typically expected that trophic level increases with increasing maturity (Jennings et al. 2002, Romanuk et al. 2011), the gut content findings obtained in this study suggested that both *A. butcheri* and *R. sarba* would decrease in trophic level with maturity due to their increasingly herbivorous feeding habits. Perhaps the consumption of larger quantities of teleost fish such as *L. presbyteroides* and the bivalve *A. senhousia* by adult *R. sarba* contributed to

the increase in trophic level of this species, whereas adult *A. butcheri* consumed very little of both of these species.

Comparison of the sparid isotopic signatures recorded in this study with those recorded for *A. butcheri* in the Walpole-Nornalup in April 2001 by Svensson et al. (2007) revealed similar isotopic compositions. However, while it was expected that the food sources in the Walpole-Nornalup would have become more marine-influenced since the early 2000s due to climate change effects (Yeoh 2018), the diets of *A. butcheri* from the present study were actually found to be slightly more freshwater-influenced than those recorded by Svensson et al. (2007). These findings could possibly reflect the opportunistic omnivorous feeding patterns of *A. butcheri* but also the short time window over which fish were sampled from both studies, which may be underplaying/skewing any real longer term trends (Peterson and Fry 1987, Michener 1994, Post 2002, Jardine et al. 2005, Katzenberg 2007).

When the current sparid isotopic signatures were considered in the broader context of those for the various other biota recorded by Svensson et al. (2007), the sparids were shown to exhibit a more terrestrial-freshwater influence than many of the marine teleosts and elasmobranchs. Moreover, they shared similar trophic levels with many of the other teleosts, tending towards the top of the food web. The isotopic signatures recorded by Svensson et al. (2007) also highlighted several potential prey sources for the sparid species, and whilst some of these prey items were similarly recorded in the gut contents during this study (e.g. *L. presbyteroides* and *Tellina* spp.), many were not (e.g. *Katelysia*, *Chaetomorpha*. and *Nassarius* spp.). However, some of these items have been recorded in the guts of *A. butcheri* by other researchers in the Walpole-Nornalup and other south-

western Australian estuarine systems, e.g. *Chaetomorpha* spp. in the Walpole-Nornalup Estuary (Sarre et al. 2000) and *Nassarius burchardii* in the Stokes Inlet (Chuwen et al. (2007)). It should also be noted that whilst Svensson et al. (2007) assessed a wide range of possible prey taxa of the three studied sparids, isotopic signatures were not reported for some key prey species identified through GCA in the current study. These included *Polisiphonia* spp., species of amphipods such as *P. excavatum*, Polychaetes as well as the seagrass, *R. megacarpa*.

Based on the trophic signatures obtained by Svensson et al. (2007) and those collected in the current study, possible predators of the three sparids included the Australian herring (*A. georgianus*) and Gummy shark (*M. antarcticus*). Australian herring have been found in previous studies to primarily feed on small crustaceans, bivalves and smaller prey attached to seagrass blades, and whilst they occasionally target small ‘bait fish’ such as whitebait, anchovies and pilchards, they are unlikely to be a key predator of the three sparids (Robertson and Hansen 1982, Smith and Brown 2014). Gummy shark, however, have been found to feed on crustaceans, cephalopods and teleosts such as wrasse (Labridae) and other smaller demersal fish species (Robertson and Hansen 1982). Whilst such predation has not been documented in the Walpole-Nornalup Estuary, it may be possible that the Gummy shark occasionally predares on the three studied sparid species. However, it is more likely that humans are the sparids’ most significant predator in the Walpole-Nornalup Estuary, combined with piscivorous birds such as cormorants, pelicans and ospreys, which are major predators of *A. butcheri* within the Swan River Estuary (Sarre and Potter 1999, Smith et al. 2009).

The overlapping distributions of the three sparids within the study zone around the lower Frankland River likely reflect a combination of influences, including physical habitat preferences, prey abundance, salinity, dissolved oxygen and tidal conditions (Blaber and Blaber 1980, Sarre and Potter 1999, Sakabe and Lyle 2010, Watsham 2016). From the research by Yeoh (2018), it was hypothesized that the shallower riverine waters would have rich sediment invertebrate communities since hypoxia was likely present in the deeper inlet waters due to the microtidal nature of the Walpole-Nornalup Estuary (Eby and Crowder 2002, Tyler et al. 2009, Tweedley et al. 2016, Warwick et al. 2018). Whilst the key factors influencing the Sparidae overlap within the Walpole-Nornalup Estuary have yet to be documented, the findings from this study indicate a moderate amount of resource overlap and hence, trophic partitioning amongst sparid size classes.

4.4 Trophic partitioning

Overall, the greatest amount of inter-specific food resource partitioning was found between *C. auratus* and the other sparids, with the gut content data reflecting a clearly more carnivorous diet for the former species and an omnivorous diet that tended more towards herbivory with size for *A. butcheri* and *R. sarba*. However, the longer-term dietary preferences reflected by the stable isotope data indicated a moderate degree of trophic overlap between *C. auratus* and particularly *R. sarba*. This could possibly be due to overlap in amphipod consumption (e.g. *P. excavatum*), but also highlights the difference in data generated by both gut content analysis and stable isotope analysis in estimating dietary characteristics.

The stable isotope analysis results also supported the observed instances of intra-specific overlap, (particularly amongst small to medium sparids), with the exception of the slight trophic separation observed between medium to larger- sized *R. sarba*. Similar instances of resource overlap were identified in sparids of the *Diplodus* genus in the Mediterranean by Sala and Ballesteros (1997), with these workers concluding that food resources were sufficiently partitioned amongst size classes to reduce intra-specific competition.

Whilst there was clearly some inter- and intra-specific dietary overlap observed between the three sparids species in this study, sustainable resource partitioning should not be does not necessarily suggest that those species feed on completely different prey types. Instead, it is far more likely to reflect differences in the proportion and relative abundance of prey consumed in order to spread predation pressures on prey sources, and allow for the sustainable coexistence of confamilial species (Platell et al. 2010, Lek et al. 2011, French et al. 2012). Thus, whilst overlap was present in the consumption of dietary items, these items were consumed to different extents, which would overall lower the effects of competition and assist in maintaining sustainable coexistence. However, there were cases of when species ate prey items in similar proportions such as the consumption of *Polisiphonia* by medium-sized *A. butcheri* and *R. sarba*.

4.5 Management implications and directions for future research

This study has found considerable dietary resource overlap both between the different Sparidae species examined, and also between different developmental stages of those species. With current and future trends indicating a drying and warming climate in south-

western Australia (Hope et al. 2015) and thus increasing salinisation of estuaries, the projected increases in abundance and diversity of marine species in these systems (Hallett et al. 2018) could lead to an increase in competition for food resources among the above sparid species in the Walpole-Nornalup Estuary.

In the near future, further reductions in river flow are also likely to increase the frequency of low dissolved oxygen concentrations in the deeper waters (Hallett et al. 2018) and may generate the widespread hypoxia that has been observed in similar systems in the south west of WA (Brearley and Hodgkin 2013, Cottingham et al. 2014, Valesini et al. 2017). Combined with projected increases in salinities, this could severely reduce the amount of habitat available for estuarine species such as *A. butcheri*. The resulting habitat compression has been correlated with declines in the growth and body condition of *A. butcheri* in the Swan-Canning Estuary (Cottingham et al. 2014, Cottingham et al. 2016, Cottingham et al. 2018). Furthermore, hypoxic events could also have drastic implications to the food abundance for the sparids in the Walpole-Nornalup Estuary (Wetz and Yoskowitz 2013, Cottingham et al. 2014). Since *A. butcheri* is confined to living within the Walpole-Nornalup Estuary, such increased resource overlap may result in substantial negative effects on this population, including contributing to reduced growth rates, poorer body condition and a decline in overall abundance (Byström et al. 1998, Fullerton et al. 2000). As *A. butcheri* are the most targeted fishery species in the estuary (Smallwood and Sumner 2007), this may result in reductions to the fishing amenity of the ecosystem (Yeoh 2018). Currently, it takes 15.5-17.5 years for *A. butcheri* in the Walpole-Nornalup Estuary to reach the MLL of 250 mm, as opposed to 3.6-12.4 years in other estuaries in the south west (Cottingham et al. 2018). Further resource competition

amongst these sparid species could hence further contribute to the already slow growth rates of *A. butcheri*.

Given the known competitive pressures between sparids in the Walpole-Nornalup Estuary, it is suggested that fisheries managers in the area closely monitor the health of the fishery in terms of sparid abundance, growth rate, size composition, body condition, etc. The WNIMP already has substantial recreational fishing pressures in contrast to many other estuarine systems in the south west, with further climate change and possible increases in sparid resource overlap, serious consequences could occur to the health and recreational fishing amenity of the system. The findings from this study will provide a baseline as to how the sparids partition their resources now to identify any changes in resource overlap/competition in the future.

4.6 Limitations and avenues for future research

In terms of study limitations, firstly it should be noted that GCA can only provide a short-term measure of the most recent meals. Since sampling was conducted over two weeks in late May and early June, this study provides only a brief snapshot of the species' true diet, which may change due to many factors such as site, season, rainfall, etc. (Sarre 1999, Platell et al. 2007). To obtain higher resolution dietary information, resampling could be conducted periodically throughout the year to assess for differences among seasons. Secondly, GCA may underestimate the contribution of relatively soft-bodied prey such as teleosts, algae and polychaetes to the diets of the three sparids, whilst over-estimating the contribution of hard-bodied and slowly digestible prey such as seagrass, crustaceans and bivalves. This is because the former items digest much faster and are typically much

harder to identify due to their more advanced state of digestion (Michener 1994, Cortés 1998, Baker et al. 2014). Thirdly, the limited number of small-sized *C. auratus* and the lack of any larger-sized individuals with identifiable prey items made dietary quantification difficult for this species.

Another assumption that must be noted is that the percentage weight contribution (%W) was used instead of the raw weight values, meaning that weight values are expressed as a proportion of the total gut weight. Using either of the two forms of weight contribution would hence have different interpretations as the raw weights would be highly variable depending on the time of capture and exact location of capture within the area of overlap. The %W was chosen instead in attempt to reduce the variability in weight measurement depending on the feeding intensity of the given fish prior to capture, since some sparids may feed heavily in the morning and others during dusk (Yeah 2018).

It should also be noted that the potential predator and prey signatures collected by Svensson et al. (2007) were sampled during 2001 and are thus may have changed in the intervening decades in response to, for example, changes in climate, salinity and species distributions.

After assessing the diets and resource partitioning of these three Sparidae species, several directions for future research have been highlighted. For instance, to understand the trophic niche and predator-prey associations to a much finer level, Bayesian mixing models could be employed to obtain a measure of the proportional contribution of prey to the consumer (Phillips et al. 2014, Stock et al. 2018).

Alternatively, one could assess the diet of *C. auratus* and *R. sarba* located in the marine waters outside of the Walpole-Nornalup Estuary to determine any shifts in diet

after marine migration. This would provide further information on the estuarine-marine connectivity and determine any shifts in diet or habitat that are crucial for supporting healthy stocks of these fishery important species (Gillanders et al. 2003; Yeoh 2018).

Dietary research could also be conducted on the three sparids in areas of the Walpole-Nornalup Estuary where they do not overlap spatially, to determine if their diet is changed when they co-occur (i.e. selective resource partitioning to reduce inter-specific competition). By doing so, this would provide an indication as to how diet changes in different locations in the estuary and provide evidence to determine if the overlap in the lower Frankland River was caused by optimal abundance.

5. Conclusions

Dietary composition differed significantly between the three Sparidae species and size classes examined, but the extent of those differences was low to moderate. Moreover, whilst small to medium *A. butcheri* had a notably greater freshwater influence in their dietary sources and fed at the highest trophic level, considerable overlap occurred in the trophic positioning of the remaining species and size classes. Collectively, these findings indicate a considerable amount of inter- and intra-specific trophic overlap, particularly between *A. butcheri* and *R. sarba* and between sparids of the largest size classes in the Walpole-Nornalup Estuary.

The first study hypothesis was supported, with *A. butcheri* and *C. auratus* being largely omnivorous and carnivorous respectively, perhaps reflecting their differing habitat preferences. Aspects of the second hypothesis were also supported, in that the greatest

ontogenetic dietary shifts occurred between small and larger fish, particularly for *R. sarba*, but other parts of this hypothesis were not supported in that dietary overlap was strongest amongst the larger sparids. This likely reflected the similar herbivorous trends of both *A. butcheri* and *R. sarba*. The third hypothesis was also partly supported in that *A. butcheri* had a more terrestrial/freshwater-derived diet compared to the greater marine influences of the other sparids. However, small to medium-sized bream had the highest in trophic level of any other species and size class, which conflicted with both the gut content results and original hypothesis and may reflect to the longer time scale over which the stable isotope signatures measured diet.

With ongoing climate change in future decades, the abundance of marine species is likely to increase in south-western Australia estuaries such as the Walpole-Nornalup, as these systems become increasingly saline. Such changes are likely to increase the level of food resource competition identified between *A. butcheri* and *R. sarba* in this study. This could have further negative impacts on the growth rates and productivity these fishery species, particularly for the estuarine *A. butcheri* which have experienced notable declines in recent decades (Cottingham et al. 2018). It is thus recommended that fisheries management closely monitors the status of the sparid fishery in the Walpole-Nornalup Estuary to help maintain both the ecological health and recreational fishing amenity of this system into the future.

6. References

- Abdulkadir, S. and M. Tsuchiya (2008). "One-step method for quantitative and qualitative analysis of fatty acids in marine animal samples." Journal of Experimental Marine Biology and Ecology **354**: 1-8.
- Able, K. W. (2005). "A re-examination of fish estuarine dependence: evidence for connectivity between estuarine and ocean habitats." Estuarine, Coastal and Shelf Science **64**: 5-17.
- ABS—Australian Bureau of Statistics (2016). 2016 Census QuickStats: Walpole. Available online at <http://www.censusdata.abs.gov.au/census_services/getproduct/census/2016/quickstat/SSC51496>.
- Anderson, M., R. N. Gorley and R. K. Clarke (2008). Permanova+ for Primer: Guide to Software and Statistical Methods, Primer-E Limited.
- Antonucci, F., C. Costa, J. Aguzzi and S. Cataudella (2009). "Ecomorphology of morpho-functional relationships in the family of sparidae: A quantitative statistic approach." Journal of Morphology **270**: 843-855.
- Auel, H., M. Harjes, R. Da Rocha, D. Stübing and W. Hagen (2002). "Lipid biomarkers indicate different ecological niches and trophic relationships of the Arctic hyperiid amphipods *Themisto abyssorum* and *T. libellula*." Polar Biology **25**: 374-383.
- Baker, R., A. Buckland and M. Sheaves (2014). "Fish gut content analysis: robust measures of diet composition." Fish and Fisheries **15**: 170-177.
- Barron, O., R. Silberstein, R. Ali, R. Donohue, D. McFarlane, P. Davies, G. Hodgson, N. Smart and M. Donn (2012). "Climate change effects on water-dependent ecosystems in south-western Australia." Journal of Hydrology **434**: 95-109.
- Berg, J. (1979). "Discussion of methods of investigating the food of fishes, with reference to a preliminary study of the prey of *Gobiusculus flavescens* (Gobiidae)." Marine biology **50**: 263-273.
- Binladen, J., M. T. P. Gilbert, J. P. Bollback, F. Panitz, C. Bendixen, R. Nielsen and E. Willerslev (2007). "The use of coded PCR primers enables high-throughput sequencing of multiple homolog amplification products by 454 parallel sequencing." PloS one **2**: e197.
- Blaber, S. (1984). "The diet, food selectivity and niche of *Rhabdosargus sarba* (Teleostei: Sparidae) in Natal estuaries." African Zoology **19**: 241-246.
- Blaber, S. and T. Blaber (1980). "Factors affecting the distribution of juvenile estuarine and inshore fish." Journal of fish biology **17**: 143-162.
- Blaber, S. J. (2008). Tropical estuarine fishes: ecology, exploration and conservation, John Wiley & Sons.
- Blaber, S. J., D. Cyrus, J.-J. Albaret, C. V. Ching, J. Day, M. Elliott, M. Fonseca, D. Hoss, J. Orensanz and I. Potter (2000). "Effects of fishing on the structure and functioning of estuarine and nearshore ecosystems." ICES Journal of Marine Science **57**: 590-602.
- Bobbie, R. J. and D. C. White (1980). "Characterization of benthic microbial community structure by high-resolution gas chromatography of fatty acid methyl esters." Applied and Environmental Microbiology **39**: 1212-1222.

- Bone, Q., N. Marshall and J. Blaxter (1995). Food and feeding. Biology of Fishes, Springer: 152-169.
- Bowen, S. H. (1996). "Quantitative description of the diet." Fisheries techniques, 2nd edition. American fisheries society, Bethesda, Maryland: 513-532.
- Brearely, A. (2005). Ernest Hodgkin's Swanland: estuaries and coastal lagoons of south-western Australia, UWA Publishing.
- Brearely, A. and E. P. Hodgkin (2013). Revisiting the Blackwood River and Hardy Inlet: 40 Years of Change, Ernest Hodgkin Trust for Estuary Education and Research.
- Burns, J., S. Trumble, M. Castellini and J. Testa (1998). "The diet of Weddell seals in McMurdo Sound, Antarctica as determined from scat collections and stable isotope analysis." Polar Biology **19**: 272-282.
- Busst, G. M. and J. R. Britton (2016). "High variability in stable isotope diet-tissue discrimination factors of two omnivorous freshwater fishes in controlled ex situ conditions." Journal of Experimental Biology: jeb. 137380.
- Byström, P., L. Persson and E. Wahlström (1998). "Competing predators and prey: juvenile bottlenecks in whole-lake experiments." Ecology **79**: 2153-2167.
- Carreon-Martinez, L., T. Johnson, S. Ludsins and D. D. Heath (2011). "Utilization of stomach content DNA to determine diet diversity in piscivorous fishes." Journal of Fish Biology **78**: 1170-1182.
- Christensen, V. and C. J. Walters (2004). "Ecopath with Ecosim: methods, capabilities and limitations." Ecological modelling **172**: 109-139.
- Chuwen, B. M., S. D. Hoeksema and I. C. Potter (2009). "Factors influencing the characteristics of the fish faunas in offshore, deeper waters of permanently-open, seasonally-open and normally-closed estuaries." Estuarine, Coastal and Shelf Science **81**: 279-295.
- Chuwen, B. M., M. E. Platell and I. C. Potter (2007). "Dietary compositions of the sparid *Acanthopagrus butcheri* in three normally closed and variably hypersaline estuaries differ markedly." Environmental Biology of Fishes **80**: 363.
- Clarke, K. and R. Green (1988). "Statistical design and analysis for a 'biological effects' study." Marine Ecology Progress Series: 213-226.
- Clarke, K. R., J. R. Tweedley and F. J. Valesini (2014). "Simple shade plots aid better long-term choices of data pre-treatment in multivariate assemblage studies." Journal of the Marine Biological Association of the United Kingdom **94**: 1-16.
- Clarke, K.R., and Gorley, R.N. (2015) PRIMER v7: User Manual/Tutorial. (PRIMER-E: Plymouth).
- Coleman, F. C., W. F. Figueira, J. S. Ueland and L. B. Crowder (2004). "The impact of United States recreational fisheries on marine fish populations." science **305**: 1958-1960.
- Colman, J. (1972). "Food of snapper, *Chrysophrys auratus* (forster), in the Hauraki Gulf, New Zealand." New Zealand journal of marine and freshwater research **6**: 221-239.
- Cook, H. W. (1991). Fatty acid desaturation and chain elongation in eucaryotes. New Comprehensive Biochemistry, Elsevier. **20**: 141-169.
- Correll, D. L. (1978). "Estuarine productivity." BioScience **28**: 646-650.

- Cortés, E. (1997). "A critical review of methods of studying fish feeding based on analysis of stomach contents: application to elasmobranch fishes." Canadian journal of fisheries and aquatic sciences **54**: 726-738.
- Cortés, E. (1998). "Methods of studying fish feeding: reply." Canadian Journal of Fisheries and Aquatic Sciences **55**: 2708.
- Costanza, R., B. Fisher, K. Mulder, S. Liu and T. Christopher (2007). "Biodiversity and ecosystem services: A multi-scale empirical study of the relationship between species richness and net primary production." Ecological economics **61**: 478-491.
- Cottingham, A., N. G. Hall, S. A. Hesp and I. C. Potter (2018). "Differential changes in production measures for an estuarine-resident sparid in deep and shallow waters following increases in hypoxia." Estuarine, Coastal and Shelf Science **202**: 155-163.
- Cottingham, A., N. G. Hall and I. C. Potter (2016a). "Factors influencing growth of *Acanthopagrus butcheri* (Sparidae) in a eutrophic estuary have changed over time." Estuarine, Coastal and Shelf Science **168**: 29-39.
- Cottingham, A., Ashworth, E., Hipsey, M., Williams, J. and Potter, I. (2016b). *Growth and body condition of Black Bream in south-western Australian estuaries have declined*. Technical report to RecfishWest, Murdoch University, Perth, Western Australia. DOI: 10.13140/RG.2.2.22018.48326.
- Cottingham, A., S. A. Hesp, N. G. Hall, M. R. Hipsey and I. C. Potter (2014). "Marked deleterious changes in the condition, growth and maturity schedules of *Acanthopagrus butcheri* (Sparidae) in an estuary reflect environmental degradation." Estuarine, Coastal and Shelf Science **149**: 109-119.
- Cottingham, A., P. Huang, M. R. Hipsey, N. G. Hall, E. Ashworth, J. Williams and I. C. Potter (2018). "Growth, condition, and maturity schedules of an estuarine fish species change in estuaries following increased hypoxia due to climate change." Ecology and evolution **8**: 7111-7130.
- Creighton, C., P. I. Boon, J. D. Brookes and M. Sheaves (2015). "Repairing Australia's estuaries for improved fisheries production—what benefits, at what cost?" Marine and Freshwater Research **66**: 493-507.
- Curnow, I. (1999). "A quality future for recreational fishing in the Gascoyne. Fisheries Management Paper no. 124." Fisheries WA, Perth.
- Dalsgaard, J., M. S. John, G. Kattner, D. Müller-Navarra and W. Hagen (2003). "Fatty acid trophic markers in the pelagic marine environment."
- David, G., R. Coutinho, I. Quagio-Grassiotto and J. Verani (2005). "The reproductive biology of *Diplodus argenteus* (Sparidae) in the coastal upwelling system of Cabo Frio, Rio de Janeiro, Brazil." African Journal of Marine Science **27**: 439-447.
- Deagle, B., S. Jarman, D. Pemberton and N. Gales (2005). "Genetic screening for prey in the gut contents from a giant squid (*Architeuthis* sp.)." Journal of Heredity **96**: 417-423.
- Deagle, B. E., R. Kirkwood and S. N. Jarman (2009). "Analysis of Australian fur seal diet by pyrosequencing prey DNA in faeces." Molecular ecology **18**: 2022-2038.
- Deeley, D. (2001). Evaluating indicators of ecological health for estuaries in southwest Australia, Murdoch University.

- DeNiro, M. J. and S. Epstein (1978). "Influence of diet on the distribution of carbon isotopes in animals." Geochimica et cosmochimica acta **42**: 495-506.
- DeNiro, M. J. and S. Epstein (1981). "Influence of diet on the distribution of nitrogen isotopes in animals." Geochimica et cosmochimica acta **45**: 341-351.
- Department of Primary Industries and Regional Development (DPIRD). (2018) Western Australian recreational fishing rules. Retrieved from <http://rules.fish.wa.gov.au>.
- Eby, L. A. and L. B. Crowder (2002). "Hypoxia-based habitat compression in the Neuse River Estuary: context-dependent shifts in behavioral avoidance thresholds." Canadian Journal of Fisheries and Aquatic Sciences **59**: 952-965.
- Elliott, M., L. Mander, K. Mazik, C. Simenstad, F. Valesini, A. Whitfield and E. Wolanski (2016). "Ecoengineering with ecohydrology: successes and failures in estuarine restoration." Estuarine, Coastal and Shelf Science **176**: 12-35.
- Elliott, M. and A. Whitfield (2011). "Challenging paradigms in estuarine ecology and management." Estuarine, Coastal and Shelf Science **94**: 306-314.
- Elton, C. S. (1927). "Animal ecology." 209.
- Fowler, A., C. Huveneers and M. Lloyd (2017). "Insights into movement behaviour of snapper (*Chrysophrys auratus*, Sparidae) from a large acoustic array." Marine and Freshwater Research **68**: 1438-1453.
- French, B., M. E. Platell, K. R. Clarke and I. C. Potter (2012). "Ranking of length-class, seasonal and regional effects on dietary compositions of the co-occurring *Pagrus auratus* (Sparidae) and *Pseudocaranx georgianus* (Carangidae)." Estuarine, Coastal and Shelf Science **115**: 309-325.
- Fry, B. (2006). Stable isotope ecology, Springer.
- Fullerton, A. H., G. A. Lamberti, D. M. Lodge and F. W. Goetz (2000). "Potential for resource competition between Eurasian ruffe and yellow perch: growth and RNA responses in laboratory experiments." Transactions of the American Fisheries Society **129**: 1331-1339.
- García-Rodríguez, M., Á. M. Fernández and A. Esteban (2006). "Characterisation, analysis and catch rates of the small-scale fisheries of the Alicante Gulf (SE Spain) over a 10 years time series." Fisheries Research **77**: 226-238.
- Gillanders, B. M., T. S. Elsdon, I. A. Halliday, G. P. Jenkins, J. B. Robins and F. J. Valesini (2011). "Potential effects of climate change on Australian estuaries and fish utilising estuaries: a review." Marine and Freshwater Research **62**: 1115-1131.
- Grandcourt, E., T. Z. Al Abdessalaam, F. Francis and A. Al Shamsi (2004). "Biology and stock assessment of the sparids, *Acanthopagrus bifasciatus* and *Argyrops spinifer* (Forsskal, 1775), in the Southern Arabian Gulf." Fisheries Research **69**: 7-20.
- Gray, C. A. and S. J. Kennelly (2003). "Catch characteristics of the commercial beach-seine fisheries in two Australian barrier estuaries." Fisheries Research **63**: 405-422.
- Grossman, G. D. (1986). "Food resource partitioning in a rocky intertidal fish assemblage." Journal of Zoology **1**: 317-355.
- Group, C. P. W., P. M. Hollingsworth, L. L. Forrest, J. L. Spouge, M. Hajibabaei, S. Ratnasingham, M. van der Bank, M. W. Chase, R. S. Cowan and D. L. Erickson

- (2009). "A DNA barcode for land plants." Proceedings of the National Academy of Sciences **106**: 12794-12797.
- Hadwen, W. L., G. L. Russell and A. H. Arthington (2007). "Gut content-and stable isotope-derived diets of four commercially and recreationally important fish species in two intermittently open estuaries." Marine and Freshwater Research **58**: 363-375.
- Hallett, C. S., A. J. Hobday, J. R. Tweedley, P. A. Thompson, K. McMahon and F. J. Valesini (2018). "Observed and predicted impacts of climate change on the estuaries of south-western Australia, a Mediterranean climate region." Regional Environmental Change: 1-17.
- Halver, J. E. and R. W. Hardy (2002). Fish nutrition, Elsevier.
- Hansson, S. (1998). "Methods of studying fish feeding: a comment." Canadian Journal of Fisheries and Aquatic Sciences **55**: 2706-2707.
- Hansson, S., J. E. Hobbie, R. Elmgren, U. Larsson, B. Fry and S. Johansson (1997). "The stable nitrogen isotope ratio as a marker of food-web interactions and fish migration." Ecology **78**: 2249-2257.
- Harasti, D., K. A. Lee, C. Gallen, J. M. Hughes and J. Stewart (2015). "Movements, home range and site fidelity of snapper (*Chrysophrys auratus*) within a temperate marine protected area." PloS one **10**: e0142454.
- Harris, D. J. (2003). "Can you bank on GenBank?" Trends in ecology & evolution **18**: 317-319.
- Hartill, B., M. Morrison, M. Smith, J. Boubée and D. Parsons (2003). "Diurnal and tidal movements of snapper (*Pagrus auratus*, Sparidae) in an estuarine environment." Marine and Freshwater Research **54**: 931-940.
- Heip, C., N. Goosen, P. Herman, J. Kromkamp, J. Middelburg and K. Soetaert (1995). "Production and consumption of biological particles in temperate tidal estuaries." Oceanography and marine biology: an annual review **33**: 1-149.
- Henry, G. and J. Lyle (2003). "The national recreational and indigenous fishing survey. Final Report to the Fisheries Research and Development Corporation, Project 99/158." NSW fisheries final report series.
- Hindell, J. (2007). "Determining patterns of use by black bream *Acanthopagrus butcheri* (Munro, 1949) of re-established habitat in a south-eastern Australian estuary." Journal of Fish Biology **71**: 1331-1346.
- Hindell, J. S., G. P. Jenkins and B. Womersley (2008). "Habitat utilisation and movement of black bream *Acanthopagrus butcheri* (Sparidae) in an Australian estuary." Marine Ecology Progress Series **366**: 219-229.
- Hodgkin, E. P. and P. Hesp (1998). "Estuaries to salt lakes: Holocene transformation of the estuarine ecosystems of south-western Australia." Marine and Freshwater Research **49**: 183-201.
- Hodgkin, E. P. and Clark, R. (1988). An Inventory of Information on the Estuaries and Coastal Lagoons of Southwestern Australia. Nornalup and Walpole Inlets. Estuaries Studies Series, Environmental Protection Authority, Western Australia.
- Hodgkin E.P. and Clark R. (1999) Nornalup and Walpole inlets and the estuaries of the Deep and Frankland Rivers. Revised Edition. Estuarine Studies Series 2, Environmental Protection Authority, Perth.

- Hoeksema, S. and I. Potter (2006). "Diel, seasonal, regional and annual variations in the characteristics of the ichthyofauna of the upper reaches of a large Australian microtidal estuary." Estuarine, Coastal and Shelf Science **67**: 503-520.
- Hope, P., D. Abbs, J. Bhend, F. Chiew, J. Church, M. Ekström, D. Kirono, A. Lenton, C. Lucas and K. McInnes (2015). "Southern and south-western flatlands cluster report." Climate change in Australia projections for Australia's natural resource management regions. CSIRO and Bureau of Meteorology, Canberra, ACT, Australia.
- Hughes, J. M., J. Stewart, B. W. Kendall and C. A. Gray (2009). "Growth and reproductive biology of tarwhine *Rhabdosargus sarba* (Sparidae) in eastern Australia." Marine and Freshwater Research **59**: 1111-1123.
- Huisman, J., MacCarthy, P., Orchard, A., Kraft, G. and Hallegraeff, G. (2006). *Algae of Australia*. Canberra: ABRS.
- Huisman, J., A. Kendrick and M. Rule (2011). "Benthic algae and seagrasses of the Walpole and Nornalup Inlets Marine Park, Western Australia." Journal of the Royal Society of Western Australia **94**: 29-44.
- Hyndes, G., M. Platell and I. Potter (1997). "Relationships between diet and body size, mouth morphology, habitat and movements of six sillaginid species in coastal waters: implications for resource partitioning." Marine Biology **128**: 585-598.
- Iverson, S. J. (2009). Tracing aquatic food webs using fatty acids: from qualitative indicators to quantitative determination. Lipids in aquatic ecosystems, Springer: 281-308.
- Iverson, S. J., C. Field, W. Don Bowen and W. Blanchard (2004). "Quantitative fatty acid signature analysis: a new method of estimating predator diets." Ecological Monographs **74**: 211-235.
- Iverson, S. J., K. J. Frost and S. L. Lang (2002). "Fat content and fatty acid composition of forage fish and invertebrates in Prince William Sound, Alaska: factors contributing to among and within species variability." Marine Ecology Progress Series **241**: 161-181.
- Jackson, A. L., R. Inger, A. C. Parnell and S. Bearhop (2011). "Comparing isotopic niche widths among and within communities: SIBER—Stable Isotope Bayesian Ellipses in R." Journal of Animal Ecology **80**: 595-602.
- Jardine, T. D., M. A. Gray, S. M. McWilliam and R. A. Cunjak (2005). "Stable isotope variability in tissues of temperate stream fishes." Transactions of the American Fisheries Society **134**: 1103-1110.
- Jarman, S. N., N. Gales, M. Tierney, P. Gill and N. Elliott (2002). "A DNA-based method for identification of krill species and its application to analysing the diet of marine vertebrate predators." Molecular Ecology **11**: 2679-2690.
- Jennings, S., J. K. Pinnegar, N. V. Polunin and K. J. Warr (2002). "Linking size-based and trophic analyses of benthic community structure." Marine Ecology Progress Series **226**: 77-85.
- Jo, H., M. Ventura, N. Vidal, J. S. Gim, T. Buchaca, L. A. Barmuta, E. Jeppesen and G. J. Joo (2016). "Discovering hidden biodiversity: the use of complementary monitoring of fish diet based on DNA barcoding in freshwater ecosystems." Ecology and evolution **6**: 219-232.

- Juanes, F., J. A. Buckel and F. S. Scharf (2002). "12 Feeding Ecology of Piscivorous Fishes." Handbook of fish biology and fisheries: Fish biology, 267.
- Käkelä, R., H. Hyvärinen and P. Vainiotalo (1993). "Fatty acid composition in liver and blubber of the Saimaa ringed seal (*Phoca hispida saimensis*) compared with that of the ringed seal (*Phoca hispida botnica*) and grey seal (*Halichoerus grypus* from the Baltic)." Comparative biochemistry and physiology. B, Comparative biochemistry **105**: 553-565.
- Katzenberg, M. A. (2007). "Stable isotope analysis: a tool for studying past diet, demography, and life history." Biological Anthropology of the Human Skeleton, Second Edition: 411-441.
- Kawaguchi, Y. and S. Nakano (2001). "Contribution of terrestrial invertebrates to the annual resource budget for salmonids in forest and grassland reaches of a headwater stream." Freshwater Biology **46**: 303-316.
- Kelly, J. R. and R. E. Scheibling (2012). "Fatty acids as dietary tracers in benthic food webs." Marine Ecology Progress Series **446**: 1-22.
- Kennish, M. J. (2002). "Environmental threats and environmental future of estuaries." Environmental conservation **29**: 78-107.
- Kirsch, P. E., S. J. Iverson, W. D. Bowen, S. R. Kerr and R. G. Ackman (1998). "Dietary effects on the fatty acid signature of whole Atlantic cod (*Gadus morhua*)." Canadian Journal of Fisheries and Aquatic Sciences **55**: 1378-1386.
- Layman, C. A., D. A. Arrington, C. G. Montaña and D. M. Post (2007). "Can stable isotope ratios provide for community-wide measures of trophic structure?" Ecology **88**: 42-48.
- Lek, E., D. Fairclough, M. Platell, K. Clarke, J. Tweedley and I. Potter (2011). "To what extent are the dietary compositions of three abundant, co-occurring labrid species different and related to latitude, habitat, body size and season?" Journal of Fish Biology **78**: 1913-1943.
- Lellis-Dibble, K. A., K. McGlynn and T. E. Bigford (2008). "Estuarine fish and shellfish species in US commercial and recreational fisheries: economic value as an incentive to protect and restore estuarine habitat."
- Lenanton, R. C. and I. C. Potter (1987). "Contribution of estuaries to commercial fisheries in temperate Western Australia and the concept of estuarine dependence." Estuaries **10**: 28.
- Linke, T. (2011). Trophic interactions among abundant members of the fish fauna in a permanently-open and a seasonally-open estuary in south-western Australia. PhD thesis. Murdoch University.
- Lukoschek, V. and M. I. McCormick (2001). "Ontogeny of diet changes in a tropical benthic carnivorous fish, *Parupeneus barberinus* (Mullidae): relationship between foraging behaviour, habitat use, jaw size, and prey selection." Marine Biology **138**: 1099-1113.
- Macdonald, J. S. and R. Green (1983). "Redundancy of variables used to describe importance of prey species in fish diets." Canadian Journal of Fisheries and Aquatic Sciences **40**: 635-637.
- Marais, J. (1980). "Aspects of food intake, food selection, and alimentary canal morphology of *Mugil cephalus* (Linnaeus, 1958), *Liza tricuspidens* (Smith,

- 1935), *L. richardsoni* (Smith, 1846), and *L. dumerili* (Steindachner, 1869)." Journal of Experimental Marine Biology and Ecology **44**: 193-209.
- McCluskey, S. M. and R. L. Lewison (2008). "Quantifying fishing effort: a synthesis of current methods and their applications." Fish and fisheries **9**: 188-200.
- McCutchan, J. H., W. M. Lewis, C. Kendall and C. C. McGrath (2003). "Variation in trophic shift for stable isotope ratios of carbon, nitrogen, and sulfur." Oikos **102**: 378-390.
- McLusky, D. S., M. Elliott and M. Elliott (2004). The estuarine ecosystem: ecology, threats and management, Oxford University Press on Demand.
- Meziane, T. and M. Tsuchiya (2000). "Fatty acids as tracers of organic matter in the sediment and food web of a mangrove/intertidal flat ecosystem, Okinawa, Japan." Marine Ecology Progress Series **200**: 49-57.
- Michener, R. (1994). "Stable isotope ratios as tracers in marine aquatic food webs." Stable isotopes in ecology and environmental science: 138-186.
- Miller, G. T. and S. Spoolman (2011). Essentials of ecology, Cengage Learning.
- Mittelbach, G. G. (1984). "Predation and resource partitioning in two sunfishes (Centrarchidae)." Ecology **65**: 499-513.
- Morison, A. K., P. C. Coutin and S. G. Robertson (1998). "Age determination of black bream, *Acanthopagrus butcheri* (Sparidae), from the Gippsland Lakes of south-eastern Australia indicates slow growth and episodic recruitment." Marine and Freshwater Research **49**: 491-498.
- New Zealand Ministry for primary Industries Fisheries Science Group (2012). Part 3: Red Gurnard to Yellow-eyed Mullet. Report from the Fisheries Assessment Plenary. Wellington.
- NLWRA—National Land and Water Resources Audit (2002). Australian Catchment, River and Estuary Assessment 2002 vol. 1. National Land and Water Resources Audit, Canberra, Australia.
- Olson, R. A., J. D. Winter, D. C. Nettles and J. M. Haynes (1988). "Resource partitioning in summer by salmonids in south-central Lake Ontario." Transactions of the American Fisheries Society **117**: 552-559.
- Paerl, H. W. (2006). "Assessing and managing nutrient-enhanced eutrophication in estuarine and coastal waters: interactive effects of human and climatic perturbations." Ecological Engineering **26**: 40-54.
- Paulin, C. D. (1990). "*Pagrus auratus*, a new combination for the species known as "snapper" in Australasian waters (Pisces: Sparidae)." New Zealand journal of Marine and Freshwater Research **24**: 259-265.
- Pauly, D., V. Christensen and C. Walters (2000). "Ecopath, Ecosim, and Ecospace as tools for evaluating ecosystem impact of fisheries." ICES journal of Marine Science **57**: 697-706.
- Pen, L. J. (1997). A systematic overview of environmental values of the wetlands, rivers and estuaries of the Busselton-Walpole region, Water and Rivers Commission.
- Peng, A. H. (2003). Comparisons of the diets of four species of sparid on the central and lower west coast of Australia, Murdoch University.
- Peterson, B. J. and B. Fry (1987). "Stable isotopes in ecosystem studies." Annual Review of Ecology and Systematics **18**: 293-320.

- Petrone, K. C., J. D. Hughes, T. G. Van Niel and R. P. Silberstein (2010). "Streamflow decline in southwestern Australia, 1950–2008." Geophysical Research Letters **37**.
- Phillips, D. L., R. Inger, S. Bearhop, A. L. Jackson, J. W. Moore, A. C. Parnell, B. X. Semmens and E. J. Ward (2014). "Best practices for use of stable isotope mixing models in food-web studies." Canadian Journal of Zoology **92**: 823-835.
- Piet, G. J. (1998). "Ecomorphology of a size-structured tropical freshwater fish community." Environmental Biology of Fishes **51**: 67-86.
- Pinkas, L., M. Ouphant and I. Iverson (1971). "Food habits of albacore, bluefin tuna, and bonito in California water." Calif. Fish, and Game, Fish Bull **152**: 1-105.
- Platell, M., H. Ang, S. Hesp and I. Potter (2007). "Comparisons between the influences of habitat, body size and season on the dietary composition of the sparid *Acanthopagrus latus* in a large marine embayment." Estuarine, Coastal and Shelf Science **72**: 626-634.
- Platell, M., S. Hesp, S. Cossington, E. Lek, S. Moore and I. Potter (2010). "Influence of selected factors on the dietary compositions of three targeted and co-occurring temperate species of reef fishes: implications for food partitioning." Journal of Fish Biology **76**: 1255-1276.
- Platell, M. and I. Potter (2001). "Partitioning of food resources amongst 18 abundant benthic carnivorous fish species in marine waters on the lower west coast of Australia." Journal of Experimental Marine Biology and Ecology **261**: 31-54.
- Polis, G. A. (1984). "Age structure component of niche width and intraspecific resource partitioning: can age groups function as ecological species?" The American Naturalist **123**: 541-564.
- Pollard, D. (1994). "A comparison of fish assemblages and fisheries in intermittently open and permanently open coastal lagoons on the south coast of New South Wales, south-eastern Australia." Estuaries **17**: 631.
- Pompanon, F., B. E. Deagle, W. O. Symondson, D. S. Brown, S. N. Jarman and P. Taberlet (2012). "Who is eating what: diet assessment using next generation sequencing." Molecular ecology **21**: 1931-1950.
- Post, D. M. (2002). "Using stable isotopes to estimate trophic position: models, methods, and assumptions." Ecology **83**: 703-718.
- Potter, I. and G. Hyndes (1994). "Composition of the fish fauna of a permanently open estuary on the southern coast of Australia, and comparisons with a nearby seasonally closed estuary." Marine Biology **121**: 199-209.
- Potter, I. and G. Hyndes (1999). "Characteristics of the ichthyofaunas of southwestern Australian estuaries, including comparisons with holarctic estuaries and estuaries elsewhere in temperate Australia: a review." Austral Ecology **24**: 395-421.
- Potter, I., G. Hyndes and F. Baronie (1993). "The fish fauna of a seasonally closed Australian estuary. Is the prevalence of estuarine-spawning species high?" Marine Biology **116**: 19-30.
- Potter, I. C., J. R. Tweedley, M. Elliott and A. K. Whitfield (2015). "The ways in which fish use estuaries: a refinement and expansion of the guild approach." Fish and Fisheries **16**: 230-239.

- Radebe, P., B. Mann, L. Beckley and A. Govender (2002). "Age and growth of *Rhabdosargus sarba* (Pisces: Sparidae), from KwaZulu-Natal, South Africa." Fisheries Research **58**: 193-201.
- Raimonet, M. and J. E. Cloern (2017). "Estuary–ocean connectivity: fast physics, slow biology." Global Change Biology **23**: 2345-2357.
- Robertson, R. C. L. A. and J. Hansen (1982). "Nearshore accumulations of detached macrophytes as nursery areas for fish." Marine Ecology Progress Series **9**: 51-57.
- Robinson, B. W., D. S. Wilson and G. O. Shea (1996). "Trade-offs of ecological specialization: an intraspecific comparison of pumpkinseed sunfish phenotypes." Ecology **77**: 170-178.
- Romanuk, T. N., A. Hayward and J. A. Hutchings (2011). "Trophic level scales positively with body size in fishes." Global Ecology and Biogeography **20**: 231-240.
- Ross, S. T. (1986). "Resource partitioning in fish assemblages: a review of field studies." Copeia: 352-388.
- Roughgarden, J. (1976). "Resource partitioning among competing species—A coevolutionary approach." Theoretical Population Biology **9**: 388-424.
- Roy, P., R. Williams, A. Jones, I. Yassini, P. Gibbs, B. Coates, R. West, P. Scanes, J. Hudson and S. Nichol (2001). "Structure and function of south-east Australian estuaries." Estuarine, Coastal and Shelf Science **53**: 351-384.
- Russell, B. (1983). "The food and feeding habits of rocky reef fish of north-eastern New Zealand." New Zealand Journal of Marine and Freshwater Research **17**: 121-145.
- Ryan, K., N. Hall, E. Lai, C. Smallwood, S. Taylor and B. Wise (2015). State-wide survey of boat-based recreational fishing in Western Australia 2013/14, Fisheries Research Division.
- Sakabe, R. and J. Lyle (2010). "The influence of tidal cycles and freshwater inflow on the distribution and movement of an estuarine resident fish *Acanthopagrus butcheri*." Journal of Fish Biology **77**: 643-660.
- Sala, E. and E. Ballesteros (1997). "Partitioning of space and food resources by three fish of the genus *Diplodus* (Sparidae) in a Mediterranean rocky infralittoral ecosystem." Marine Ecology Progress Series **152**: 273-283.
- Santini, F., G. Carnevale and L. Sorenson (2014). "First multi-locus timetree of seabreams and porgies (Percomorpha: Sparidae)." Italian Journal of Zoology **81**: 55-71.
- Sarre, G., M. Platell and I. Potter (2000). "Do the dietary compositions of *Acanthopagrus butcheri* in four estuaries and a coastal lake vary with body size and season and within and amongst these water bodies?" Journal of Fish Biology **56**: 103-122.
- Sarre, G. and I. Potter (1999). "Comparisons between the reproductive biology of black bream *Acanthopagrus butcheri* (Teleostei: Sparidae) in four estuaries with widely differing characteristics." International Journal of Salt Lake Research **8**: 179-210.
- Sarre, G. A. (1999). Age compositions, growth rates, reproductive biology and diets of the black bream *Acanthopagrus butcheri* in four estuaries and a coastal saline lake in south-western Australia, Murdoch University.

- Sarre, G. A. and I. C. Potter (2000). "Variation in age compositions and growth rates of *Acanthopagrus butcheri* (Sparidae) among estuaries: some possible contributing factors." Fishery Bulletin **98**: 785-799.
- Schoener, T. W. (1974). "Resource partitioning in ecological communities." Science **185**: 27-39.
- Semeniuk, V., C. Semeniuk, C. Tauss, J. Unno and M. Brocx (2011). Walpole and Nornalup Inlets: landforms, stratigraphy, evolution, hydrology, water quality, biota, and geoheritage, Wetlands Research Association.
- Shipley, L. A., J. S. Forbey and B. D. Moore (2009). "Revisiting the dietary niche: when is a mammalian herbivore a specialist?" Integrative and Comparative Biology **49**: 274-290.
- Silberstein, R., S. Aryal, J. Durrant, M. Pearcey, M. Braccia, S. Charles, L. Boniecka, G. Hodgson, M. Bari and N. Viney (2012). "Climate change and runoff in south-western Australia." Journal of Hydrology **475**: 441-455.
- Skrzypek, G. (2013). "Normalization procedures and reference material selection in stable HCNOS isotope analyses: an overview." Analytical and Bioanalytical Chemistry **405**: 2815-2823.
- Skuilason, S. and T. Smith (1995). "Resource polymorphism in vertebrates." Trends in Ecology and Evolution **10**: 366-370.
- Smallwood, C. B. and N. R. Sumner (2007). A 12-month survey of recreational estuarine fishing in the South Coast bioregion of Western Australia during 2002/03, Citeseer.
- Smith, K. and J. Brown (2014). Biological synopsis of Australian herring (*Arripis georgianus*), Fisheries Research Division, Western Australian Fisheries and Marine Research Laboratories.
- Smith, K., J. Norriss and J. Brown (2009). "Population growth and mass mortality of an estuarine fish, *Acanthopagrus butcheri*, unlawfully introduced into an inland lake." Aquatic Conservation: Marine and Freshwater Ecosystems **19**: 4-13.
- Smith, K. A. and K. Smith (2006). Review of fishery resources and status of key fishery stocks in the Swan-Canning Estuary, Fisheries Research Division, Western Australian Fisheries and Marine Research Laboratories.
- Smith, S. J., S. J. Iverson and W. Bowen (1997). "Fatty acid signatures and classification trees: new tools for investigating the foraging ecology of seals." Canadian Journal of Fisheries and Aquatic Sciences **54**: 1377-1386.
- Somerfield, P. J. and K. R. Clarke (2013). "Inverse analysis in non-parametric multivariate analyses: distinguishing groups of associated species which covary coherently across samples." Journal of Experimental Marine Biology and Ecology **449**: 261-273.
- St John, M. and T. Lund (1996). "Lipid biomarkers: linking the utilization of frontal plankton biomass to enhanced condition of juvenile North Sea cod." Marine Ecology Progress Series **131**: 75-85.
- Stock, B. C., A. L. Jackson, E. J. Ward, A. C. Parnell, D. L. Phillips and B. X. Semmens (2018). Analyzing mixing systems using a new generation of Bayesian tracer mixing models, PeerJ Preprints.

- Stoner, A. W. and R. J. Livingston (1984). "Ontogenetic patterns in diet and feeding morphology in sympatric sparid fishes from seagrass meadows." Copeia: 174-187.
- Svanbäck, R. and D. I. Bolnick (2007). "Intraspecific competition drives increased resource use diversity within a natural population." Proceedings of the Royal Society of London B: Biological Sciences **274**: 839-844.
- Svanbäck, R. and P. Eklöv (2003). "Morphology dependent foraging efficiency in perch: a trade-off for ecological specialization?" Oikos **102**: 273-284.
- Svensson, C. J., G. A. Hyndes and P. S. Lavery (2007). "Food web analysis in two permanently open temperate estuaries: consequences of saltmarsh loss?" Marine Environmental Research **64**: 286-304.
- Tieszen, L. L., T. W. Boutton, K. G. Tesdahl and N. A. Slade (1983). "Fractionation and turnover of stable carbon isotopes in animal tissues: implications for $\delta^{13}\text{C}$ analysis of diet." Oecologia **57**: 32-37.
- Traugott, M., S. Kamenova, L. Ruess, J. Seeber and M. Plantegenest (2013). Empirically characterising trophic networks: what emerging DNA-based methods, stable isotope and fatty acid analyses can offer. Advances in Ecological Research, Elsevier. **49**: 177-224.
- Tweedley, J. R., C. S. Hallett, R. M. Warwick, K. R. Clarke and I. C. Potter (2016). "The hypoxia that developed in a microtidal estuary following an extreme storm produced dramatic changes in the benthos." Marine and Freshwater Research **67**: 327-341.
- Tyler, R. M., D. C. Brady and T. E. Targett (2009). "Temporal and spatial dynamics of diel-cycling hypoxia in estuarine tributaries." Estuaries and Coasts **32**: 123-145.
- Ulanowicz, R. E. (2004). "Quantitative methods for ecological network analysis." Computational Biology and Chemistry **28**: 321-339.
- Usmar, N. (2012). "Ontogenetic diet shifts in snapper (*Pagrus auratus*: Sparidae) within a New Zealand estuary." New Zealand Journal of Marine and Freshwater Research **46**: 31-46.
- Valesini, F., A. Cottingham, C. Hallett and K. Clarke (2017). "Interdecadal changes in the community, population and individual levels of the fish fauna of an extensively modified estuary." Journal of fish biology **90**: 1734-1767.
- Wainwright, P. C. and B. A. Richard (1995). "Predicting patterns of prey use from morphology of fishes." Environmental Biology of Fishes **44**: 97-113.
- Wakefield, C. B. (2006). Latitudinal and temporal comparisons of the reproductive biology and growth of snapper, *Pagrus auratus* (Sparidae), in Western Australia, Murdoch University.
- Wang, Y., S. Li, L. Duan and Y. Liu (2012). "Fishery policy exploration in the Pearl River Estuary based on an Ecosim model." Ecological modelling **230**: 34-43.
- Ward, R. D., R. Hanner and P. D. Hebert (2009). "The campaign to DNA barcode all fishes, FISH-BOL." Journal of fish biology **74**: 329-356.
- Warwick, R. M., J. R. Tweedley and I. C. Potter (2018). "Microtidal estuaries warrant special management measures that recognise their critical vulnerability to pollution and climate change." Marine pollution bulletin **135**: 41-46.

- Water Corporation WA (2018). Perth Rainfall | Today, Annual and Average Statistics - Retrieved from <https://www.watercorporation.com.au/water-supply/rainfall-and-dams/rainfall>.
- Watsham, J. (2016). The influence of environmental factors on the habitat use of Black Bream *Acanthopagrus butcheri* and the implications of artificial oxygenation in the Swan-Canning Estuary. Honours thesis. Murdoch University.
- Wetz, M. S. and D. W. Yoskowitz (2013). "An 'extreme' future for estuaries? Effects of extreme climatic events on estuarine water quality and ecology." Marine Pollution Bulletin **69**: 7-18.
- Whitfield, A. (2016). "Biomass and productivity of fishes in estuaries: a South African case study." Journal of fish biology **89**: 1917-1930.
- Whitfield, A. and M. Elliott (2011). "1.07-Ecosystem and biotic classifications of estuaries and coasts." Treatise on estuarine and coastal science: 99-124.
- Whittaker, R. H., S. A. Levin and R. B. Root (1973). "Niche, habitat, and ecotope." The American Naturalist **107**: 321-338.
- Wolanski, E. and M. Elliott (2015). Estuarine ecohydrology: an introduction, Elsevier.
- Woodward, G. and A. G. Hildrew (2002). "Body-size determinants of niche overlap and intraguild predation within a complex food web." Journal of Animal Ecology **71**: 1063-1074.
- Xie, S., Y. Cui and Z. Li (2001). "Dietary-morphological relationships of fishes in Liangzi Lake, China." Journal of fish biology **58**: 1714-1729.
- Yeoh D. (2018). Understanding the dynamics of fish ecology and movements: implications for management of a temperate estuarine marine park. Unpublished PhD thesis. Murdoch University.
- Zaret, T. M. and A. S. Rand (1971). "Competition in tropical stream fishes: support for the competitive exclusion principle." Ecology **52**: 336-342.