Population biology and ecology of the greenback flounder (*Rhombosolea tapirina*) in the Coorong estuary, South Australia

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Summary

The greenback flounder, *Rhombosolea tapirina*, occurs in estuaries and coastal waters throughout south-eastern Australia and New Zealand where it supports commercial and recreational fisheries. The aim of this study was to inform on the population biology of the species in the Coorong estuary, South Australia, and the processes that contribute to variation in population structure and abundance. In particular, this thesis considered aspects of the adult biology of the species in context of the significant environmental changes that occurred in mid-2010, associated with the delivery of drought-breaking freshwater flows to the system.

Analysis of the long-term chronology of fishery production of *R. tapirina* in the Coorong estuary indicated extreme inter-annual variation in population abundance and dispersion. This variation was driven partly by fluctuations in the magnitude of freshwater inflow, because large areas of high quality, sheltered and food-rich estuarine habitat are only available during and immediately after years of increased freshwater inflow.

A direct fish ageing protocol, based on interpreting the macrostructure of otoliths of *R. tapirina*, was developed and provided estimates of age-based data. Males and females grew rapidly, particularly during the first 12 months of life. There were no differences in growth between the sexes, despite the strong bias in numbers toward female fish. Spatial differences in growth were marginal. The population was truncated in age, consisting of only a few young age classes. This likely related to ontogenetic migration to the marine environment and the removal of larger individuals by the commercial fishery.

Assessment of the reproductive biology of *R. tapirina* indicated that it is a multiple batch spawner, with asynchronous oocyte development, indeterminate fecundity, and early maturation. Spawning occurred from March to October in each year, and was most frequent from May to August, i.e. when water temperature was lowest. Comparison of the reproductive biology between areas of contrasting salinity regimes showed strong similarities in spawning activity.

Assessment of the feeding ecology of *R. tapirina* using a combination of gut contents analysis and stable isotope analysis indicated that the species is a carnivorous, generalist feeder that exploited food resources from a wide array of taxonomic groups. The main

components of the diet were polychaetes, gammarid amphipods, and bivalve siphons. The species exhibited a distinct ontogenetic trophic shift that likely related to changes in feeding morphology. There were distinct differences in diet before and after the commencement of drought-breaking freshwater inflows which likely related to changes in prey availability.

Analysis of the movement patterns of adult *R. tapirina*, based on acoustic telemetry, demonstrated the individualistic and often highly transient nature of the movement behaviour of individuals. This included its ability to undertake regular movements over 10s of kilometres between the estuary and the sea. Tagged fish were detected over a large part of the system, i.e. from near the Murray Mouth to approximately 45 km away in the inner estuary, but they showed a strong preference for 'estuarine habitat' in the inner estuary. Furthermore, they exhibited a distinct diurnal shift in fine-scale habitat use, with individuals occupying deeper habitats during the day and shallower areas during the night.

The findings of this research indicated that the previously-documented variation in fishery production for *R. tapirina* is likely related to the movement of individuals from the estuary to the marine environment during their second or third years of life; fluctuations in the amount of estuarine habitat available within the Coorong as a consequence of extreme variability in freshwater inflows to the system; and the removal of individuals by the commercial fishery. The sustainability of this population would depend on an ecosystem-based management approach that ensured the delivery of seasonal freshwater inflows to the estuary; maintained connectivity between the estuarine and marine environments; and managed the commercial fishery, particularly during periods of poor environmental condition.

Declaration

I certify that this thesis does not incorporate without acknowledgement any material previously submitted for a degree or diploma in any university; and that to the best of my knowledge and belief it does not contain any material previously published or written by another person except where due reference is made in the text.

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Jason Earl

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Cover photo: Australian National Fish Collection, CSIRO.

Publications

A revised version of Chapter 3 from this thesis is published in the New Zealand Journal of Marine and Freshwater Research. The complete reference for the research article is:

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1   General Introduction

1.1   Estuaries

Estuaries are dynamic, transitional environments where freshwater from land drainage mixes with the saline waters of the ocean to create some of the most biologically productive areas on Earth (Levin et al. 2001; Kennish 2002). Estuaries provide important ecosystem services through the provision of sheltered habitat and relatively high productivity, which support a complexity of interacting biological and ecological processes that involve organisms from marine, freshwater and terrestrial environments (Worm et al. 2006; Barbier et al. 2010).

The ecological functioning of estuaries is dependent on adequate, seasonal inflows of freshwater (Whitfield and Bruton 1989; Abrantes and Sheaves 2010). Seasonal cycles of temperature and river discharge stimulate recurrent changes in primary and secondary productivity (Kennish et al. 2008), flush accumulated sediments, form salinity gradients that increase habitat complexity, and maintain connection with the sea which prevent salinity and temperature extremes and facilitate the movement of organisms between marine, estuarine and freshwater habitats (Schlacher and Wooldridge 1996). Such benefits are reflected by the highly abundant and diverse communities of flora and fauna that commonly occur in estuaries including numerous species of seagrasses, birds, fish, marine mammals and invertebrates (Beck et al. 2001; Elliott and Hemingway 2002; Shaughnessy et al. 2012).

Despite their ecological significance, estuaries are among the most heavily exploited and degraded natural systems globally (Lotze et al. 2006; Worm et al. 2006). Whilst the impacts of a warming climate are becoming evident (Hobday and Lough 2011), the environmental deterioration of estuaries relates also to other anthropogenic influences that include pollution, river regulation, sediment input, and encroaching coastal development, all of which can cause loss and alteration of habitat (Kennish et al. 2008). Furthermore, the impacts of overfishing on populations of individual species and estuarine ecosystems are increasing (Kennish 2002).

1.2   Fish in estuaries

Estuaries often support more abundant and diverse assemblages of fish than adjacent riverine and coastal environments (Elliott and Hemingway 2002). This may relate to a complexity of factors

such as: higher food availability due to increased primary and secondary productivity; different water temperature regimes; and/or protection from predation by stenohaline species and storm events (Beck et al. 2001). However, the way that different fish species use estuaries can be quite different: some species exploit these environments as juveniles; some enter intermittently as adults from the ocean or river; while others complete their entire life cycle within estuaries (Potter et al. 1990).

Knowledge of the roles of estuaries in the life-cycles of different fish species has enabled the development of numerous functional guilds, which group species based on their modes of feeding, reproductive strategies and the relevant life-history stages (Claridge et al. 1986; Potter and Hyndes 1999; Whitfield 1999; Thiel et al. 2003; Elliott et al. 2007; Franco et al. 2008). The most comprehensive functional guild for estuarine-associated fish species was established by Elliott et al. (2007) and considers the seasonal and spatial occurrence of species in marine, freshwater and estuarine environments, as well as their biological attributes (Table 1.1). For example, fish species that spend all or the majority of their lives in estuaries are recognised as either 'estuarine residents' or 'estuarine migrants', i.e. species that have larval stages completed outside the estuary or that are also represented by discrete marine or freshwater populations. Elliott et al. (2007) identified 14 different functional groups and highlighted the diversity of life-history patterns of species associated with estuaries.

Understanding the relationship between estuarine habitats and many fish species is limited by a lack of information on their life-histories and population dynamics. Such information is imperative for managing the effects of climate change and human activities, such as river regulation and commercial fishing, for the different estuarine fish species and to ensure the sustainability of their populations in these environmentally-complex systems.

1.3   Fisheries in estuaries

Fishing is an integral human activity in the coastal zone, particularly in estuaries, due to the diverse and highly abundant assemblages of fish that occur in these systems and their ease of access (Blaber 1997). Given their well-defined roles in the life cycles of fish species (Elliot et al. 2007), estuaries are critically important for fisheries production (Potter et al. 1990; Whitfield 1999). In Australia, ~75 % of species targeted by commercial fisheries spend parts of their life cycles within estuarine and associated environments (Creighton 2013). Most of these fisheries are either fully or over-exploited (Lenanton and Potter 1987; Blaber et al. 2000).

Table 1.1 Functional groups of fishes based on estuarine use (taken from Elliot et al. 2007).

Functional group	Definition
Estuarine species	This category can be subdivided into: 1. Estuarine residents: estuarine species capable of completing their entire life cycle within the estuarine environment. 2. Estuarine migrants: Estuarine species that have larval stages of their life cycle completed outside the estuary or are also represented by discrete marine or freshwater populations.
Marine migrants	Species that spawn at sea and often enter estuaries in large numbers and particularly as juveniles. Some of these species are highly euryhaline and move throughout the full length of the estuary. This category can be subdivided into: 1. Marine estuarine-opportunist: marine species that regularly enter estuaries in substantial numbers, particularly as juveniles, but use, to varying degrees, nearshore marine waters as an alternative habitat. 2. Marine estuarine dependent: marine species that require sheltered estuarine habitats as juveniles but live along coasts where there are no such habitats and these species are thus dependent on the habitats of that type that are present in estuaries.
Marine stragglers	Species that spawn at sea and typically enter estuaries only in low numbers and occur most frequently in the lower reaches where salinities are approximately 35 PSU. These species are often stenohaline and associated with coastal marine waters.
Freshwater migrants	Freshwater species found regularly and in moderate numbers in estuaries and whose distribution can extend beyond the oligohaline sections of these systems.
Freshwater stragglers	Freshwater species found in low numbers in estuaries and whose distribution is usually limited to the low salinity, upper reaches of estuaries.
Anadromous	Species that undergo their greatest growth at sea and which, prior to the attainment of maturity, migrate into rivers where spawning subsequently occurs.
Semi-anadromous	Species whose spawning migration from the sea extends only as far as the upper estuary rather than going into freshwater.
Catadromous	Species that spend all of their trophic life in freshwater and which subsequently migrate out to sea to spawn.
Semi-catadromous	Species whose spawning run extends only to estuarine areas rather than the marine environment.
Amphidromous	Species which migrate between the sea and freshwater and in which the migration in neither direction is related to reproduction.

Fishing can have significant impacts on the assemblage structure and abundance of estuarine fish species (Chesney et al. 2000; Elliott and Hemingway 2002). It has been implicated in the decline of numerous estuarine-associated species worldwide, including some that are not directly targeted (Blaber et al. 2000; Silberschneider et al. 2008). Effects of sustained fishing on populations can include declines in population size and changes size and age composition as well as sex ratio (Fromentin and Fonteneau 2001; Worm and Myers 2004). Such changes can greatly affect the

resilience of populations, as there are fewer remnant, larger and older mature fish, to ensure the population can overcome years of poor recruitment (Longhurst 2002; Fowler et al. 2008; Planque et al. 2010). Furthermore, overfishing can contribute to imbalances in biotic community structure and ecosystem functioning (Jennings and Kaiser 1998).

For managers of exploited estuarine fish populations, one of the main challenges is to separate the effects of fishing, variation in freshwater flows and other processes that influence the size of the population (Blaber et al. 2000). This requires a comprehensive understanding of the population biology of individual species.

1.4   Coorong estuary

The Coorong estuary is situated at the terminus of Murray-Darling River system in southern Australia. The extensive network of catchments and tributaries of the Murray-Darling Basin (MDB) drains an area of 1,073,000 km^2, making it Australia's largest river system. The Murray River discharges into a shallow expansive lake system, comprised of Lakes Alexandrina and Albert, before flowing into the Coorong and finally the Southern Ocean via the Murray Mouth (Figure 1.1). The original Murray River estuary covered 660 km^2 and included the Lakes and Coorong (Walker 2006). However, in the late 1930s, five barrages with a total length of 7.6 km were constructed between the Lakes and Coorong to prevent saltwater intrusion into the Lakes and to maintain stable freshwater storage in the lower Murray River to support agriculture and urban development. The remnant estuary is now only 11 % of its original size (Leblanc et al. 2012) and comprises a shallow lagoon that extends south-east from the near the Murray Mouth, parallel to the coast for approximately 130 km (Webster 2010) (Figure 1.1).

Since river irrigation began early in the 20$^{\text{th}}$ century, river regulation and water extraction has reduced median annual freshwater flow into the Coorong estuary by 71 % (CSIRO 2008), with strong inter-annual variation in discharge (Puckridge et al. 1998) (Figure 1.2). Furthermore, the river now ceases to flow through the Murray Mouth 40 % of the time, compared to 1 % under natural unregulated conditions (CSIRO 2008). Due to the recent Millennium Drought (Leblanc et al. 2012), freshwater inflows to the Coorong estuary fell further from 2002 to 2010, with no flows from 2007 to August 2010. The lack of flow contributed to an extensive salinity gradient which extended south-east from the mouth of the estuary (Webster 2010) and an overall decline in the ecological condition of the system (Lester and Fairweather 2009). In 2010, freshwater flows recommenced and continued throughout 2011, 2012 and 2013.
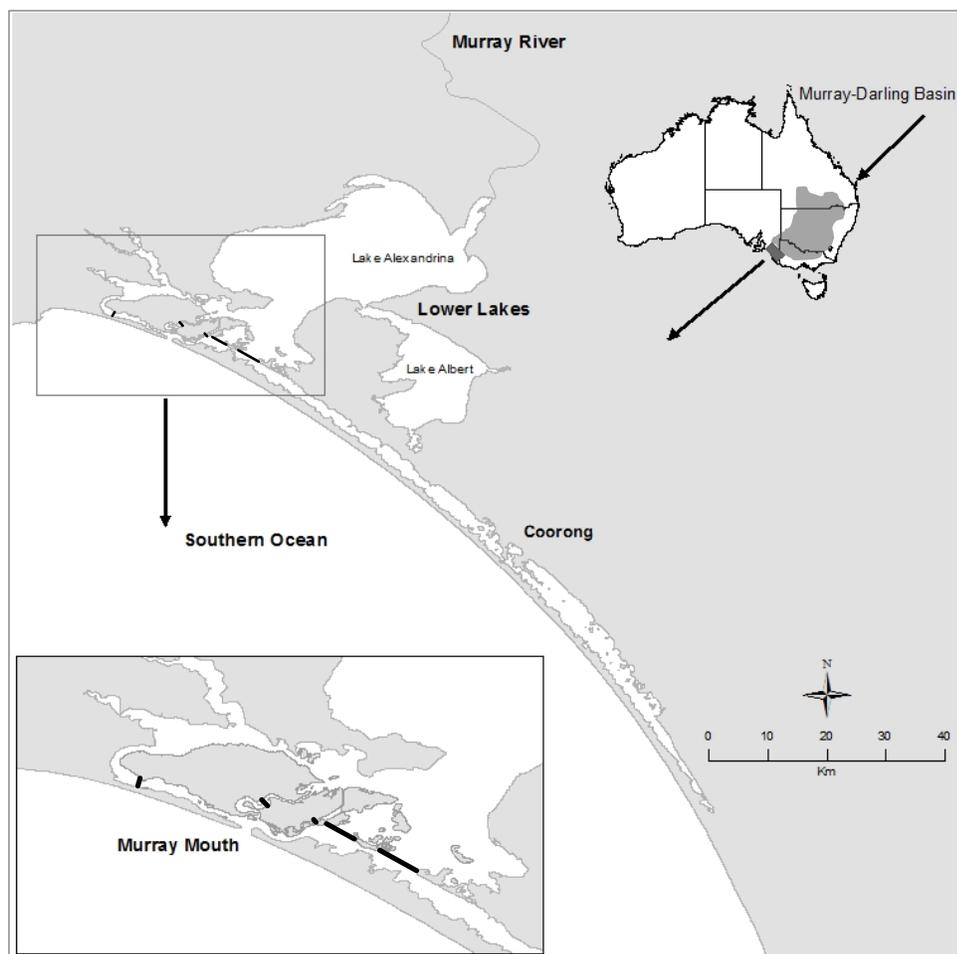
Figure 1.1 Map of the Coorong estuary and lower Murray River including Lakes Alexandrina and Albert. Inset shows the Murray Mouth and the five barrages (black lines) between the estuary and Lake Alexandrina.

The Coorong estuary represents the largest area of estuarine habitat in temperate Australia and is recognised internationally for its remarkable physical and biological diversity that includes some of the world's largest populations of migratory shorebirds (Phillips and Muller 2006), abundant assemblages of invertebrates (Dittmann et al. 2012) and an expanding colony of New Zealand fur seals (Shaughnessy et al. 2012). This unique coastal ecosystem also supports significant populations of numerous species of fish, some of which are exploited by commercial and recreational fisheries, with mulloway (*Argyrosomus japonicus*), yelloweye mullet (*Aldrichetta forsteri*), black bream (*Acanthopagrus butcheri*) and greenback flounder (*Rhombosolea tapirina*) being particularly important (Ferguson 2012).
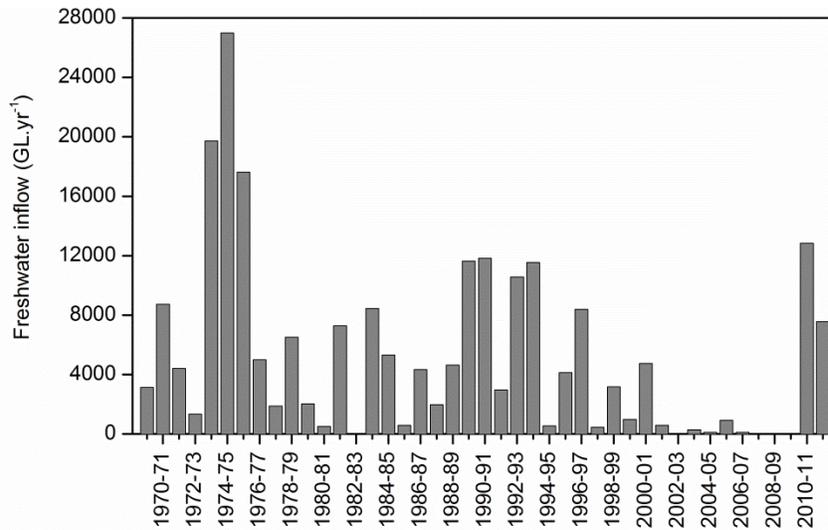
Figure 1.2 Estimates of total annual freshwater discharge through the barrages and into the Coorong estuary from 1969/70 to 2011/12 (data source: Murray-Darling Basin Authority 2013).

1.5   Greenback flounder (*Rhombosolea tapirina*, Günther 1862)

Fishes of the order Pleuronectiformes are characterised by an adult form that has an asymmetrically flattened body shape with both eyes on the same side of the head (Chapleau 1993). This order, commonly known as flatfishes or flounders, is represented by 14 families, some of which have eyes on the left side of the head while others have them on the right (Gomon et al. 2008). The most common pleuronectid in Australian waters is the greenback flounder, *Rhombosolea tapirina* (van den Enden et al. 2000). *R. tapirina* is a member of Rhombosoleidae, otherwise known as the family of Austral right-eyed flounders, which consists of 9 genera and 19 species, most of which are endemic to the southern coasts of Australia and New Zealand (Gomon et al. 2008). *R. tapirina* is the only one of four species in the Australasian genus *Rhombosolea* (Gomon et al. 2008) that occurs in Australian waters. The key diagnostic features for identification of *R. tapirina* are: the absence of ventral fins on the lower body surface; and a distinctive pointed snout (Last et al. 1983). *R. tapirina* attains a maximum total length of 50 cm (Sutton et al. 2010) and is the only flatfish in southern Australia that is large and abundant enough to be commercially exploited.

The distribution of *R. tapirina* in Australia extends from southern New South Wales and Tasmania, to south-eastern Western Australia (Kailola et al. 1993). They also occur in New Zealand, around the Auckland and Campbell Islands (Gomon et al. 2008). Adult *R. tapirina* are most common over sand, silt and mud substrates in bays, estuaries and coastal waters to depths of

100 m, while juveniles are more common in shallower water, often less than 1 m deep, and prefer unvegetated sand and mudflat habitats (Edgar and Shaw 1995a; Jenkins et al. 1997).

R. tapirina support commercial and recreational fisheries in southern New Zealand and in south-eastern Australia, with small catches also taken in Western Australia (Kailola et al. 1993; Froese and Pauly 2013). In South Australia, almost all commercial catches of *R. tapirina* are taken by the Lakes and Coorong Fishery (LCF) which operates in, and adjacent to the Coorong estuary. The LCF is a multi-species, multi-gear fishery that targets *R. tapirina* using large-mesh gill nets (mesh > 115 mm) (Ferguson 2012). Long-term statistics for the LCF indicate extreme inter-annual variation in population abundance, including historically low catches during the last decade (Figure 1.3), and this has caused concern about the sustainability of this fishery (Ferguson 2007).
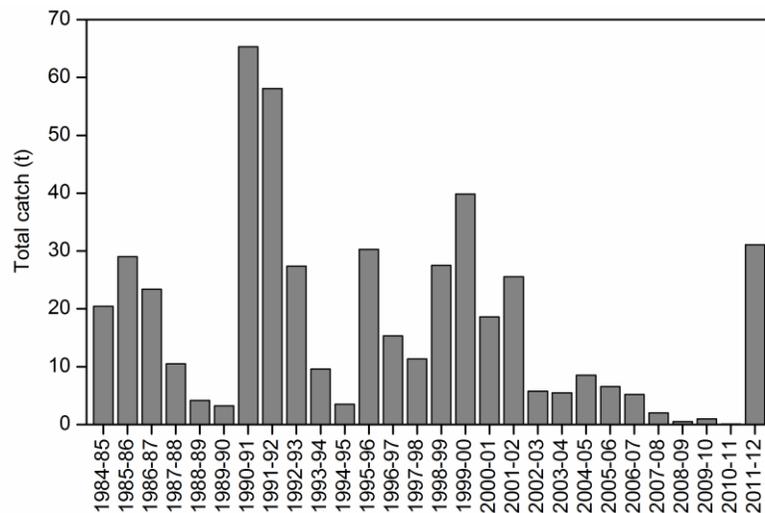
Figure 1.3 Estimates of total annual catch of *R. tapirina* by the Lakes and Coorong Fishery from 1984/85 to 2011/12 (Ferguson 2012).

Despite the economic significance of *R. tapirina*, fundamental information on its biology and ecology is not available, thus limiting the capacity to make informed management decisions. Early studies on the species provided descriptions of population genetic structure (van den Enden et al. 2000), its early life characteristics (Jenkins 1987a; Jenkins 1987b; Stewart and Jenkins 1991; May and Jenkins 1992; Shaw and Jenkins 1992; Hart 1994), and its potential for aquaculture (Hart 1994; Barnett and Pankhurst 1999; Cox and Pankhurst 2000; Pankhurst and Fitzgibbon 2006). Some information is also available on the ecology of populations in Tasmania (Kurth 1957; Crawford 1984), which is hundreds of kilometres from the Coorong estuary. However, it is unknown whether the findings of those studies on the population biology of *R. tapirina* are applicable to the population in South Australia, because, in general, flatfish display large

differences in their basic biology over relatively small spatial scales (Gibson 2005). For these reasons, this study on the population biology and ecology of *R. tapirina* in the Coorong estuary was undertaken.

1.6   Objectives

The primary aim of this study was to inform on the population biology and ecology of the greenback flounder, *Rhombosolea tapirina*, in the Coorong estuary, South Australia, and the factors and processes that regulate population structure and abundance. In particular, this thesis considers some spatial aspects of the adult biology of the species in the context of significant environmental changes that occurred in mid-2010. The specific objectives were:

1. to describe the long-term patterns of exploitation of *R. tapirina* in the Coorong estuary and to consider the potential influence of freshwater inflow on population abundance;

2. to provide an assessment of the usefulness of the otoliths of *R. tapirina* for ageing and estimates of age-based data for the Coorong population, including growth;

3. to describe the reproductive characteristics and temporal variation in gonad development and spawning activity of *R. tapirina* and to relate these to water temperature and salinity;

4. to determine the dietary composition of *R. tapirina* in the Coorong estuary to provide a better understanding of its feeding habits and how these may be affected by flow-related changes in the environment; and

5. to determine the spatial and temporal patterns of movement, residency and habitat use by *R. tapirina* in the Coorong estuary during a period of relatively high freshwater discharge.

1.7   Thesis structure

This thesis is presented in manuscript format. The thesis is organised into five data chapters (Chapter 2 - 6) which address the various characteristics of the population biology and ecology of *R. tapirina* in the Coorong estuary including abundance and distribution, age, growth and population characteristics, reproduction, diet, and movement and habitat use. Chapter 7 provides a synthesis of the findings, an overview of the population dynamics for *R. tapirina* in the Coorong estuary and how they are influenced by the environment, and directions for future research.

2 Historical patterns of exploitation on greenback flounder (*Rhombosolea tapirina*) in relation to freshwater inflow to the Coorong estuary, South Australia

2.1 Introduction

Estuaries often support a more abundant and diverse assemblage of fish than adjacent coastal environments (Elliott and Hemingway 2002). This may relate to higher food availability due to higher primary and secondary productivity and/or protection from predation by stenohaline species or larger individuals of the same species (Beck et al. 2001). Many fish species utilise estuarine habitats as juveniles, or intermittently as adults, while others complete their entire life cycles within the estuarine environment (Potter et al. 1990). Numerous estuarine fish species have declined in abundance, due to the disruption to their population dynamics by a range of natural and anthropogenic stressors such as river regulation and fishing pressure (Anderson et al. 2008; Hidalgo et al. 2011).

Estuarine fish populations can be highly dynamic as a consequence of variable environmental conditions that occur in estuaries (Whitfield and Elliott 2002). However, for populations of exploited species, the selective harvesting of large numbers of fish can also influence population size (Sadovy and Cheung 2003). Effects of sustained fishing on populations can include decreases in abundance, changes in size composition and truncation of age structures (Fromentin and Fonteneau 2001; Worm and Myers 2004). Such changes can greatly affect the resilience of populations, as there are fewer remnant larger and older fish, to help the population overcome years of poor recruitment (Longhurst 2002; Fowler et al. 2008; Planque et al. 2010). It is for this reason that fishing has been implicated in the decline of numerous estuarine-associated species worldwide (Blaber et al. 2000; Silberschneider et al. 2008). Therefore, understanding the population dynamics of an exploited species must take into consideration the historical patterns in commercial fishing.

The Coorong estuary is situated at the terminus of the Murray-Darling River system in South Australia. This estuarine system comprises a long and narrow lagoon that extends south-east from the river mouth, approximately 110 km, and represents one of the largest areas of estuarine habitat

in temperate Australia. The original estuary covered 660 km^2 and included the brackish Lakes Alexandrina and Albert and the Coorong lagoons. In the early1940s, a series of barrages were constructed between the Lakes and the Coorong, reducing the estuary to 11% of its original size (Leblanc et al. 2012). Since then, water extraction for agriculture and human consumption has reduced mean annual flow to 40 % of natural levels (Kingsford *et al*. 2011). The regulation of remaining flows, has led to inconsistent patterns of inflow to the estuary, including prolonged periods of no flow. From 2001 to 2010, the Murray River system experienced its worst drought in recorded history, which had serious consequences for the abundance and distribution of numerous finfish species that use the estuary (Ye et al. 2013). However, for most species, the influence of such an unpredictable and highly regulated freshwater inflow regime on their population dynamics is poorly understood.

The Coorong estuary is the geographic centre of the Australian distribution of greenback flounder, *Rhombosolea tapirina,* a species known for its strong association with estuaries (Kurth 1957; Crawford 1984). *R. tapirina* is a medium-sized, demersal flatfish of the family Pleuronectidae (Gomon et al. 2008). Of the four species in the temperate Australasian genus *Rhombosolea, R. tapirina* is the only species that is sufficiently large and abundant to be commercially exploited (Edgar 2000). This species occupies estuaries and inshore coastal environments in southern Australia and New Zealand where it supports numerous local commercial and recreational fisheries (Kailola et al. 1993; Froese and Pauly 2013). In South Australia, almost all catches are taken by the multi-species Lakes and Coorong Fishery (LCF) which operates in the Coorong estuary and adjacent areas (Ferguson 2007). Fishers in the LCF harvest *R. tapirina* using mainly large-mesh gill nets, whilst recreational fishers also target this species in shallow water at night using spot-lights and spears (Kailola et al. 1993).

In South Australia, variability in the annual catch of *R. tapirina* from the Coorong estuary has been attributed to variability in freshwater inflows (Hall 1984). The aim of this chapter was to describe the long-term patterns of exploitation on *R. tapirina* in the Coorong estuary and to consider the potential influence of freshwater inflow on population abundance for this species. To achieve these aims, the specific objectives were to: (1) compile data on fishery catches to document the history of exploitation in the Coorong estuary; (2) describe the temporal variation in commercial fishing statistics, as the only available indicators of the relative abundance of *R. tapirina*; (3) describe the spatial patterns of relative abundance of *R. tapirina* based on fishery catch and effort data; (4) describe the temporal variation in freshwater flow to the Coorong

estuary; and (5) examine the influence of freshwater flow on the temporal and spatial patterns of commercial catch and CPUE for *R. tapirina*.

2.2   Materials and methods

2.2.1   *Compilation of commercial fishery data*

Estimates of annual commercial catch of *R. tapirina* in South Australia were reconstructed from several historical sources dating back to 1936/37 (i.e. 1st July 1936 to 30th June 1937). From that year until 1957/58, the only data available were estimates of the total weight of *R. tapirina* processed annually at South Australia's main fish market, as presented in the annual reports of the South Australian Fisheries and Game Department. During that period, it was not compulsory for fishers to transport their entire catch to this market each day, thus these data are only indicative of the minimum State-wide commercial catch at that time. No market records were available for the period from 1946/47 to 1950/51. From 1957/58 to 1975/76, quantities of *R. tapirina* processed at the South Australian fish market were not recorded. Rather they were categorised into a broad group of 'mixed species', which included numerous other species that were presumably processed in low quantities. As such, no catch estimates were available for this period.

From 1976/77 to 1983/84 total catch data for *R. tapirina* were recorded by commercial fishers and reported in annual reports of the South Australian Fishing Industry Council Inc. However, information on the location from which the catches were taken was not available. Since July 1 1984, commercial catch and effort data have been collected by fishers in the LCF completing a research logbook for each fishing day. Daily catch and effort data include estimates of catch (kg), effort (fisher days) for targeted and non-targeted species, and the spatial reporting area (SRA, *n* = 13) in which the fishing activity took place (Figure 2.1). These data have been submitted to SARDI Aquatic Sciences on a monthly basis and have been maintained in a catch and effort database.

2.2.2   *Analysis of commercial fishery data*

Estimates of total catch were collated by financial year for the period from 1936/37 to 2011/12, excluding the two periods from 1946/47 to 1950/51 and from 1957/58 - 1975/76. Total catch included non-targeted catch, taken as by-catch from fishing operations that targeted other species. Estimates of targeted catch, targeted effort and targeted catch-per-unit-effort (CPUE: kg.fisher day^{-1}) for the period from 1984/85 to 2010/11 were collated by financial year. Annual CPUE was

estimated by dividing the targeted catch for each year by the targeted effort (fisher days) used to land that catch.
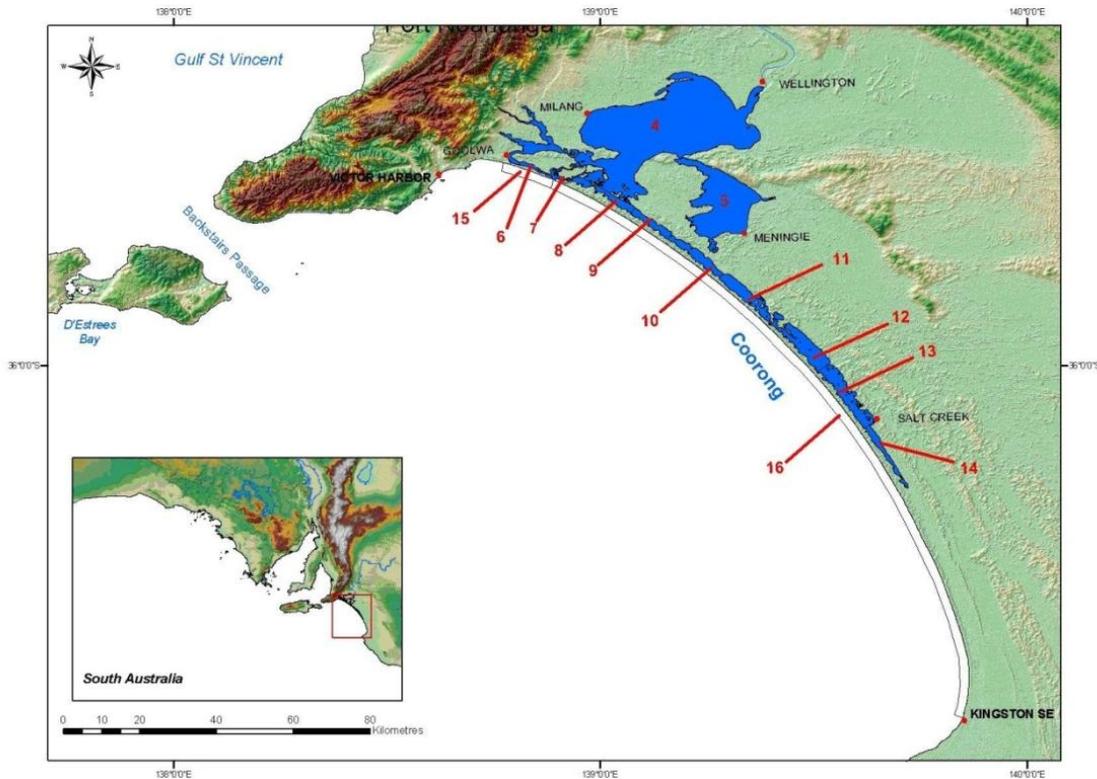
Figure 2.1 Map of the Coorong estuary indicating the spatial reporting areas (4 – 16) for the Lakes and Coorong Fishery.

Estimates of annual targeted catch, effort and CPUE were examined to describe the temporal and spatial variation in the relative abundance of *R. tapirina* available to the LCF. These data were examined at two spatial scales: (1) the entire area exploited by the LCF, i.e. Coorong estuary and adjacent areas; and (2) two broad areas inside the estuary, defined as the 'Estuary', which comprised SRAs 6, 7 and 8; and 'North Coorong', which comprised SRAs 9, 10 and 11 (Figure 2.1). Targeted catch was compared to total catch, targeted effort and CPUE using linear regression ($\alpha = 0.05$).

The contributions made by the Estuary and North Coorong and all other areas of the LCF to the total annual catch in each year from 1984/85 to 2011/12 were also presented to describe the spatial variation in catch among years. Estimates of catch for individual SRAs were not presented due to data confidentiality arrangements, as they involved fewer than five fishers in some years.

2.2.3 Relationship between freshwater inflow and commercial fishery data

Here, "inflow" refers to freshwater from the Murray River entering the Coorong estuary through the barrage system. Estimated mean annual freshwater inflow (GL) to the Coorong estuary was obtained from the regression-based Murray hydrological model (MSM BIGMOD, Murray-Darling Basin Commission) for the period from 1969/70 to 2011/12. All inflow data were reported in financial years, to align with the fishery catch and effort data. Relationships between freshwater inflow and targeted catch and CPUE for the Estuary and North Coorong from 1984/85 to 2011/12 were examined using linear regression ($\alpha = 0.05$). Further analyses were done using time lags of one and two years to assess the influence of inflow on relative population abundance following changes in freshwater inflow using linear regression. All statistical analyses were done using the statistical package SPSS v.19.0 for Windows (SPSS Inc., Chicago, Illinois, USA).

2.3   Results

2.3.1 Analysis of the commercial fishery data

Historical trends in total catch

Between 1936/37 and 1945/46, estimates of annual catch of *R. tapirina* taken from South Australian waters were low, peaking at 1.4 t in 1936/37 (Figure 2.2). The next available estimates of annual catch were for the years between 1951/52 and 1957/58, which ranged from 0.9 to 31.8 $t.yr^{-1}$. The absence of fish market records from 1957/58 to 1975/76 is likely to reflect low catches during this period. In 1976/77 and 1977/78, reported catches exceeded 231 $t.yr^{-1}$, and to this day, represent the highest annual catches of *R. tapirina* taken by the LCF. From that time, catches fluctuated periodically until the early 2000s, but were lower and ranged from 3.2 to 65.3 $t.yr^{-1}$. The high catches reported in some years through this period were typically followed by gradual declines in subsequent years. Annual catches during the 2000s were low and represented the longest period of low catches yet recorded in the fishery. These catches ranged from 0.5 to 8.5 $t.yr^{-1}$, before declining to only 0.1 t in 2010/11. Catch increased to 31 t in 2011/12.
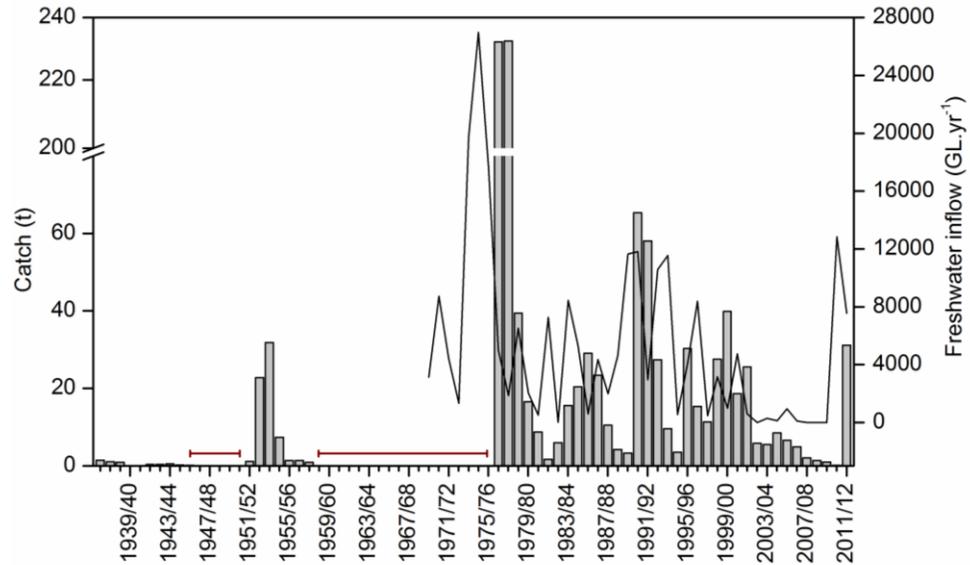
Figure 2.2 Estimates of total annual catch of *R. tapirina* in South Australia from 1936/37 to 2011/12. Horizontal capped red lines indicate the two periods for which catch estimates were unavailable. Also shown are estimates of total annual freshwater inflow since 1969/70.

Targeted catch, effort and CPUE

Estimates of annual targeted catch fluctuated periodically from 1984/85 to 2011/12 and ranged between $0.1 – 47.5$ t.yr^{-1} (Figure 2.3A). They were low during the late 1980s, dropping to less than 1 t in 1988/89 and 1989/90. In the following year, targeted catch increased to 47.5 t, before catches declined in subsequent years to 0.8 t in 1994/95. From 1995/96 until 2001/02, targeted catch was highly variable and ranged from 6.3 to 31.1 t. From 2002/03 to 2009/10, annual catch did not exceed 4 t and was less than 0.2 t in 2008/09 and 2009/10. In 2011/12, targeted catch increased to 19.8 t.

Targeted catch was generally a strong indicator of total catch in each year (Table 2.1). From 1984/85 to 2011/12, targeted catch explained 93 % of the variability in total catch, indicating that when the catch of *R. tapirina* was high, the species was targeted, but when it was low it was not heavily targeted.

Estimates of annual targeted effort fluctuated periodically among years from 1984/85 to 2011/12 and were strongly correlated with targeted catches (Table 2.1; Figure 2.3B). Targeted effort was highest in the early 1990s, with a peak of 1577 fisher days in 1991/92. It decreased to 100 fisher days in 1994/95 and was highly variable during the subsequent 12 years, ranging from 160 to 860 fisher days. In 2007/08, effort declined to 64 fisher days, and did not exceed 9 fisher days for

any year from 2008/09 to 2010/11. It then increased to 571 fisher days in 2011/12. The strong positive relationship between targeted catch and effort (Table 2.1), suggests that the amount of *R. tapirina* being caught was dependent on the amount of effort commercial fishers spent targeting the species. Hence, years of low targeted effort likely corresponded to years when the biomass of fish available to the fishery was low.
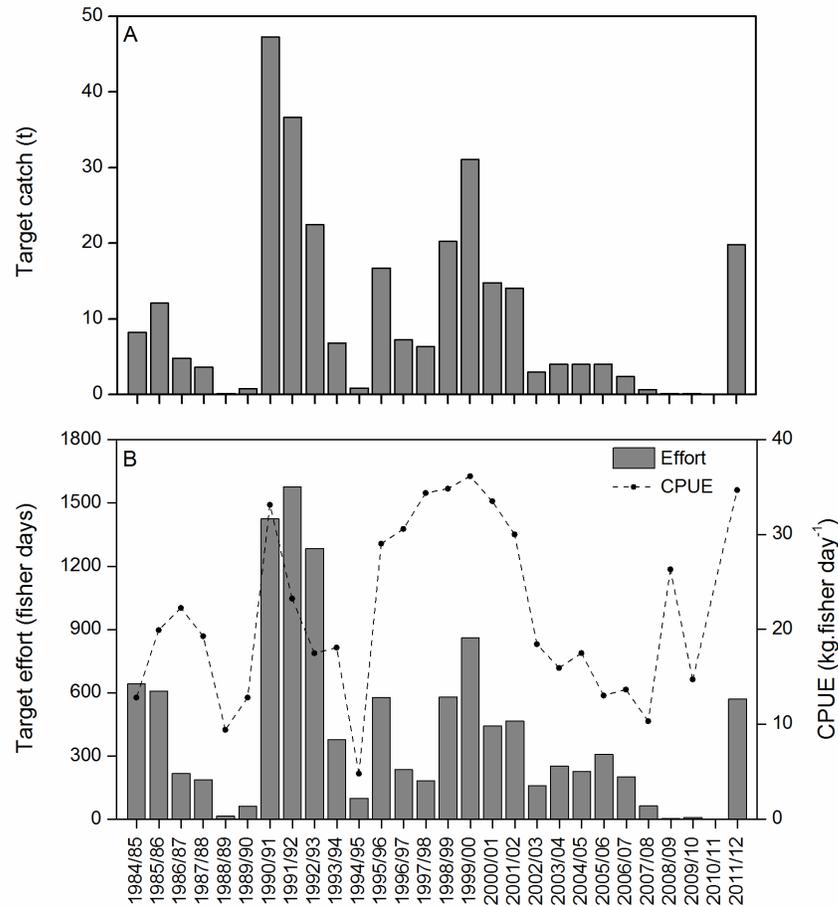
Figure 2.3 Annual targeted catch and effort for *R. tapirina* for the LCF from 1984/85 to 2011/12. (A) Targeted catch (t); (B) targeted effort (fisher days) and CPUE (fisher days.kg^{-1}).

CPUE was highly variable between 1984/85 and 2011/12, ranging from 8.8 to 36.2 kg.fisher day^{-1} (Figure 2.3B). CPUE rose to a peak of 33 kg.fisher day^{-1} in 1990/91 and a sustained peak of > 29 kg.fisher day^{-1} between 1995/96 to 2001/02. From then, CPUE declined to 2007/08, before rising to 26.3 kg.fisher day^{-1} in 2008/09 and remaining relatively high to 2011/12. CPUE was positively correlated with annual targeted catch, but not with annual target effort (Table 2.1).

Table 2.1 Summary of regression analyses performed on total catch, targeted catch, targeted effort, CPUE data for the LCF for *R. tapirina* for the period from 1984/85 to 2011/12. The coefficients of determination (r^2) are shown with their *p* values (significant, $^*\alpha = 0.05$, $^{**}\alpha = 0.005$, ^{ns} non-significant).

		Regression statistics	
Independent variable	**Dependent variable**	r^2	*p* value
targeted catch	total catch	0.93	$<0.001^{**}$
targeted effort	targeted catch	0.77	$<0.001^{**}$
targeted catch	CPUE	0.39	$<0.001^{**}$
targeted effort	CPUE	0.11	0.103^{ns}

Spatial distribution of catches

From 1984/85 to 2011/12, catches were reported from all 13 SRAs in the LCF. On average, 84 % of annual catch was taken from three SRAs, i.e. SRA 8, 9 and 10 that accounted for 29 %, 23 % and 32 %, respectively (Figure 2.4A). Most of the remaining catch was taken from SRA 11 which accounted for an average of 9 % of the total catch. Small contributions were also made by areas near the mouth of the estuary, i.e. SRA 6 and 7, whilst catches from the southern Coorong (SRA 12, 13 and 14), and from the nearshore marine environment (SRA 15 and 16) were minimal.

 From 1984/85 to 2004/05, catches were dominated by contributions from North Coorong, i.e. SRAs 9 – 11, with most of the remainder taken from the Estuary, i.e. SRAs 6 – 8 (Figure 2.4B). However, from 2004/05, the fishery contracted spatially and contributions from the North Coorong gradually decreased, while those from the Estuary increased. From 2008/09 to 2010/11, almost all catches were taken from the Estuary. This situation changed dramatically in 2011/12, when most of the catch was taken from North Coorong.

Spatial trends in targeted catch, effort and CPUE

Estuary: Targeted annual catches from the Estuary were highly variable from 1984/85 to 2011/12 and relatively low compared to the targeted annual catches of the LCF (Figure 2.5A). In the 5-year period to 1989/90, fishers landed just 3.5 t as targeted catch, including several years when no catches were taken. Targeted catch increased to 8.6 t in 1991/92, before declining and remaining less than 3.2 t until 1998/99. After a peak of 6 t in 1999/00, catches declined and were less 0.2 t in each year from 2007/08 to 2011/12.
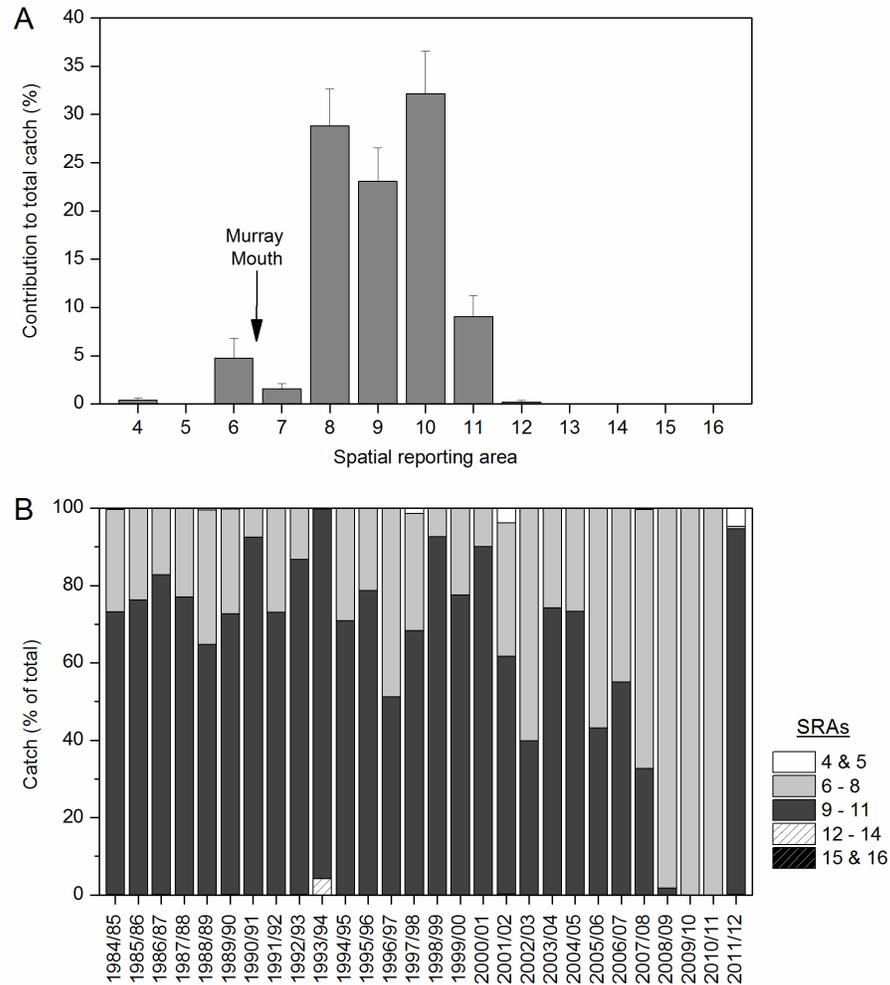
Figure 2.4 (A) Average (±S.E.) annual contribution of each spatial reporting area (SRA) to the total annual catch in the LCF from 1984/85 to 2011/12. (B) Catches from five broad groups of SRAs within the LCF, expressed as a percentage of the total annual catch.

Targeted fishing effort in the Estuary followed a similar temporal pattern to targeted catch (Figure 2.5B). It peaked at 475 fisher days in 1991/92 and then declined and remained relatively low until 2011/12. This included several years when no effort was recorded and a 5-year period from 2007/08 when effort was less than 4 fisher days. Targeted effort was a strong indicator of targeted catch (Table 2.2), which suggests that when abundances of *R. tapirina* were high, the species was targeted, but when low it was generally captured in gear used to target other species.

Estimates of CPUE were highly variable among years (Figure 2.5B). It was relatively low prior to 1995/96, then peaked at 44 kg.fisher day^{-1} in 2000/01 (Figure 2.5B). From then, CPUE declined and remained relatively low to 2011/12. There was a marginal positive relationship between CPUE and targeted catch, whilst there was no relationship with targeted effort (Table 2.2).

Table 2.2 Summary of regression analyses performed on targeted catch, targeted effort, CPUE data for *R. tapirina* harvested from the Estuary and North Coorong for the period from 1984/85 to 2011/12. The coefficients of determination (r^2) are shown with their *p* values (significant, [*]$\alpha = 0.05$, [**]$\alpha = 0.005$, [ns] non-significant).

Area	Independent variable	Dependent variable	Regression statistics	
			r^2	*p* value
Estuary	targeted effort	targeted catch	0.62	<0.001[**]
	targeted catch	CPUE	0.19	0.049[*]
	targeted effort	CPUE	0.00	0.937[ns]
North Coorong	targeted effort	targeted catch	0.80	<0.001[**]
	targeted catch	CPUE	0.58	<0.001[**]
	targeted effort	CPUE	0.35	<0.001[**]

North Coorong: Targeted catches fluctuated considerably among years and were, on average, 81 % higher than in the Estuary in each year (Figure 2.5C). Catches were highest in the early 1990s, peaked at 43.4 t in 1990/91, and then fluctuated periodically until 2007/08, averaging 9.3 t.yr^{-1}. No targeted fishing occurred from 2008/09 to 2010/11, but resumed in 2011/12 producing 19.5 t of *R. tapirina*.

Targeted catches were strongly correlated with targeted effort (Figure 2.5D; Table 2.2), which was, on average, 73 % higher than for the Estuary. Effort peaked at 1262 fisher days in 1990/91, declined annually until 1994/95, before increasing to a secondary peak of 663 fisher days in 1999/00. From then it declined in most years until 2008/09 when fishing for *R. tapirina* ceased. In 2011/12, 561 fisher days of targeted effort was recorded.

CPUE in North Coorong fluctuated periodically through several cycles that each spanned several years (Figure 2.5D). From 1984/85 to 2008/09, it ranged between 8 and 41 kg.fisher day^{-1}, with a peak in 1990/91 and a period of relatively high CPUE between 1995/96 and 2001/02. This peak period was followed by a gradual decline in most years until the period from 2008/09 to 2010/11 when annual CPUE was zero. The resumption of targeted fishing in 2011/12 coincided with the relatively high CPUE of 35 kg.fisher day^{-1}.

Similar to the Estuary, targeted catch and CPUE were strongly related in the North Coorong (Table 2.2), indicating that fishery production was driven by variation in fishable biomass, rather than fishing effort. However, there was also a moderate positive relationship between targeted effort and catch rate indicating that fishing effort increased during periods of higher population biomass.
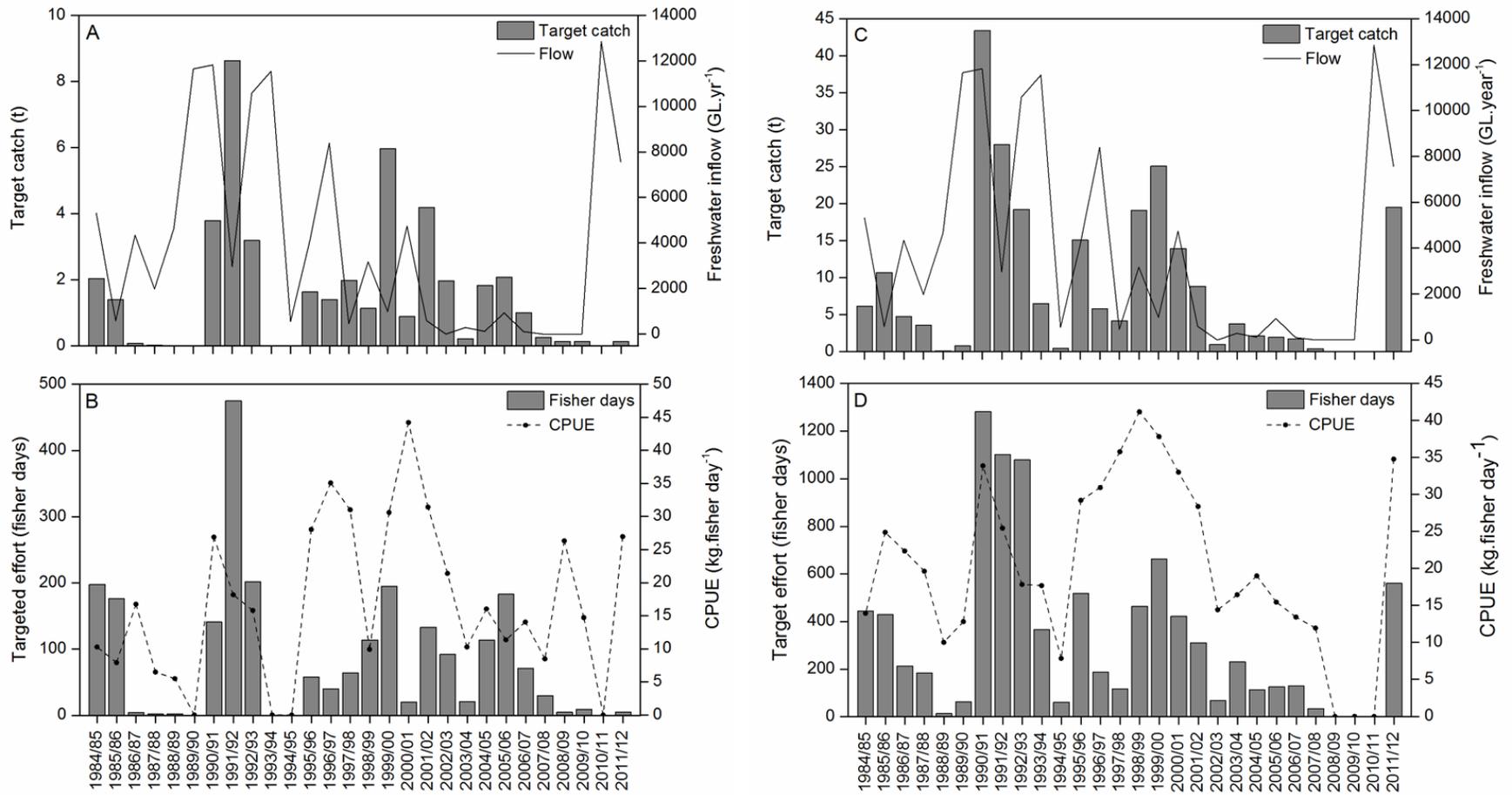
Figure 2.5 Annual targeted catch and effort for *R. tapirina* for the Estuary (left, A & B) and North Coorong (right, C & D) from 1984/85 to 2011/12. (A & C) Targeted catch (t) and freshwater inflow (GL.year^{-1}) to the Coorong estuary; (B & D) targeted effort (fisher days) and CPUE (kg.fisher day^{-1}). Dashed lines link successive data points and do not infer linear trends.

2.3.2   *Temporal variation in freshwater flows*

Since 1969/70, mean annual freshwater inflows to the Coorong estuary have been highly variable among years (Figure 2.2). Inflow was highest in the mid-1970s when it averaged 21,439 GL.yr^{-1}. From then, it did not exceed 12,000 GL.yr^{-1} again until 2010/11. Minimal inflow in 1980/81, led to the temporary closure of the Murray Mouth, before it was naturally re-opened the following year. Inflows were > 10,000 GL.yr^{-1} in several years during the early 1990s and then declined to 2000/01. From then until 2009/10, severe drought in the Murray-Darling Basin contributed to a prolonged period of low flow, including no flow in 2002/03 and from 2007/08 to 2009/10. As a consequence, a dredging program was established in 2002 to keep the mouth of the estuary open. Dredging continued until 2010/11, when major flooding throughout the Murray-Darling Basin resulted in an annual freshwater flow of 12,849 GL, which restored the natural connection between the estuary and the sea.

2.3.3   *Influence of freshwater flow on fishery catch and CPUE*

For the North Coorong, i.e. the area that accounted for the majority of the catch of *R. tapirina*, there was a significant positive correlation between targeted catch and freshwater inflow, for lags of zero, one and two years, although in each case the proportion of variance (r^2) explained was quite low (Table 2.3). There were no such significant relationships for CPUE. For the Estuary, there were no correlations between targeted catch or CPUE with inflow.

2.4   Discussion

Historical catch estimates dating back to the 1930s were compiled and examined to provide a chronology of the long-term variability in exploitation of *R. tapirina* in the Coorong estuary. While *R. tapirina* was harvested in the Coorong by the indigenous Ngarrindjeri people prior to European settlement in 1836 (Jenkin 1979), the earliest estimates of catch of *R. tapirina* by the commercial fishing sector were from the late 1930s and 1940s. Catches during this period were low which may reflect low fishing effort through World War II and/or low population abundance during the period of barrage construction. Annual catches increased during the 1950s, whilst the lack of catch information from the 1960s and early-1970s suggests that catches were low throughout this period. An unprecedented increase in catch in the mid-1970s culminated in the fishery's highest recorded catches in 1976/77 and 1977/78. From then until the early 2000s, catches fluctuated periodically, but were generally much lower. They declined further through the 2000s and were minimal by the end of that decade, before increasing in 2011/12 to a level not

seen since the 1990s. Temporal variation in annual catch indicated that the biomass of *R. tapirina* harvested from the Coorong estuary was highly variable among years.

Table 2.3 Summary of regression analyses performed on freshwater inflow data and targeted catch and CPUE data for *R. tapirina* harvested from the Estuary and North Coorong from 1984/85 to 2011/12. For the independent variable, subscript (t) is the time lag in years. The coefficients of determination (r^2) are shown with their *p* values (significant, $^*\alpha = 0.05$, ^{ns} non-significant).

Area	Independent variable	Dependent variable	Regression statistics	
			r^2	*p* value
Estuary	inflow	targeted catch	0.02	0.522^{ns}
	inflow $_{(t-1year)}$	targeted catch	0.14	0.061^{ns}
	inflow $_{(t-2years)}$	targeted catch	0.14	0.059^{ns}
	inflow	CPUE	0.00	0.992^{ns}
	inflow $_{(t-1year)}$	CPUE	0.01	0.669^{ns}
	inflow $_{(t-2years)}$	CPUE	0.00	0.897^{ns}
North Coorong	inflow	targeted catch	0.14	0.048^{*}
	inflow $_{(t-1year)}$	targeted catch	0.23	0.012^{*}
	inflow $_{(t-2years)}$	targeted catch	0.2	0.022^{*}
	inflow	CPUE	0.05	0.254^{ns}
	inflow $_{(t-1year)}$	CPUE	0.13	0.064^{ns}
	inflow $_{(t-2years)}$	CPUE	0.05	0.276^{ns}

Annual estimates of CPUE provided the only available measure of relative population abundance for *R. tapirina*. As such, temporal trends in CPUE were examined to help understand the high inter-annual variation in fishery production in the Coorong estuary. Whilst CPUE data were only available from 1984/85 onwards, they indicated that relative population abundance was highly variable among years during that period. The synchronous relationship between annual catch and CPUE indicated that catch was also a strong indicator of population abundance. There was no such relationship between CPUE and effort, indicating that fishery production was driven mostly by the availability of fish, rather than fishing effort. Hence, variation in annual catch and CPUE is likely to reflect variation in population abundance and biomass of *R. tapirina* in the system.

The highest ever recorded catches of *R. tapirina* from the mid-1970s were likely a result of an historic peak in population abundance. This peak in catch, as well as several other smaller peaks reported in some years afterwards, was typically followed by abrupt declines in subsequent years. The long-term periodic nature of the variation in population abundance suggests the likelihood that high fishing mortality experienced in the peak fishing years affected the population dynamics of the species. Whilst similar cyclic-temporal patterns in population size are not uncommon for

exploited estuarine fish populations (Livingston 1997; Loneragan and Bunn 1999; Ferguson et al. 2008; Gillson et al. 2009), the generalised boom-and-bust phenomenon is commonly a consequence of the complex interplay between key demographic processes (i.e. movement), environment conditions and variation in exploitation (Lobon-Cervia 2009).

Variation in the spatial distribution of catch was examined in order to better understand the possible mechanisms driving the inter-annual variation in population abundance for *R. tapirina* in the Coorong estuary. Whilst catches were reported from all 13 reporting areas of the LCF in the past 29 years, most of the catch in each year was taken from areas in North Coorong, with smaller catches taken from the Estuary. However, during the drought years, i.e. from 2001 to 2010, the distribution of the population contracted towards the Murray Mouth and from 2008/09 to 2010/11, almost all catches were taken from this area. The increase in catch from the North Coorong in 2011/12 indicates that a significant proportion of the population returned to this area following a year of relatively high freshwater inflow.

Annual estimates of CPUE were also higher in the North Coorong compared to the Estuary, which further supports the hypothesis that population abundance was generally highest in that part of the system. However, uncertainty exists around the use of CPUE as a measure of relative abundance of *R. tapirina* in the Coorong estuary because the area available for fishing varies substantially between years. This spatial variability is likely to affect the catchability of the species, thus confounding CPUE as an indicator of population abundance.

Nonetheless, spatial variation in abundance of *R. tapirina*, derived from temporal trends in catch and CPUE, did not appear to relate to preferences for specific habitat types, as each area comprises shallow and protected sandy and/or muddy unvegetated habitats (Gilbertson 1981). However, the hydrological characteristics of each area can be quite different due to their respective proximities to the Murray Mouth and the barrages (Geddes 1987). In the Estuary, conditions are dynamic and relatively unstable due to fluctuations in the magnitude, frequency and duration of freshwater inflows, as well as the intrusion of seawater pushing through the Murray Mouth (Geddes 1987; Webster 2010). Whereas, North Coorong represents the interface between the hypersaline southern Coorong and the variable conditions of the Estuary, and it is rarely directly affected by the intrusion of seawater. Knowledge of how the population dynamics of *R. tapirina* are influenced by freshwater inflow and associated hydrological conditions is essential to help explain the spatial and temporal variation in population size for this species.

Freshwater inflow to the Coorong estuary did occur, but was nevertheless variable in all years between 1968/69 and 2001/02, with no inflow from 2007/08 to 2009/10. The impacts of a lack of consistent seasonal freshwater input and lengthy periods of no inflow on estuarine fish populations are well established (Beck et al. 2001). Such impacts generally relate to loss and/or alteration of habitat and trophic resources in response to changes in salinity (Whitfield 2005). The geographic characteristics of the Coorong estuary make the majority of the system highly susceptible to hypersalinity, especially during periods of minimal and/or no inflow (Geddes 1987). During such times, seawater exchange through the narrowed Murray Mouth is the only means of water regulation and this is generally limited to areas within approximately 15 km of the Murray Mouth (Kingsford et al. 2011). As a consequence, waters in the remaining areas of the system often stagnate and become hypersaline, thus reducing the quality and availability of habitat for many finfish species, including *R. tapirina*.

The influence of variation in freshwater inflow on the relative abundance and distribution of *R. tapirina* in the Coorong estuary was assessed. No detectable relationship was found between inflow and CPUE in each area. This may have been partly attributable to changes in catchability of *R. tapirina* due to changes in the area available for fishing. Furthermore, there was no correlation between catch and freshwater inflow in the Estuary, which may have been partly due to the likely abrupt decline in salinity in this area during periods of high freshwater discharge (Geddes 1987). However, for the North Coorong, i.e. the area from which most of the catch of *R. tapirina* was taken, there was a significant correlation between catch and inflow in the same year and one and two years later which suggest freshwater inflow had a positive influence on population abundance in this area.

Numerous studies have demonstrated the benefits of freshwater inflow to the quality and availability of habitat for fish in temperate estuaries (Loneragan et al. 1987; Loneragan and Potter 1990; Kimmerer 2002; Whitfield 2005). However, different fish species utilise estuaries in different ways and so the effect of freshwater discharge will vary among species. Despite a lack of information on the population dynamics of *R. tapirina,* it is probable that this species, like numerous other estuarine species, is highly responsive to environmental change (Murawski and Finn 1988; Sheaves et al. 1999; Whitfield and Elliott 2002). Spatio-temporal fluctuations in the abundance of *R. tapirina* suggests that in years of high freshwater discharge and those immediately after, the majority of the population occupies habitats in the North Coorong, i.e. where brackish conditions dominate and potential prey items are generally most abundant

(Dittmann et al. 2012). In contrast, during prolonged periods of low or no inflow, the population contracts to the vicinity of the Murray Mouth, where conditions are regulated by tidal movement (Webster 2010). This contraction is likely to increase competition for food resources in the remaining area of available habitat (Schlacher and Wooldridge 1996; Whitfield 2005; Bennett 2012); enhance the vulnerability of the population to fishing (Loneragan and Bunn 1999; Robins et al. 2005); and encourage individuals to emigrate from the estuary to other marine environments that offer better conditions (Sheaves et al. 1999). Furthermore, loss of estuarine habitat during years of low and no inflow may contribute to poor recruitment and possibly recruitment failure (Ferguson et al. 2008). Whilst differentiating between the numerous plausible explanations for the high inter-annual variation in population abundance for *R. tapirina* in the Coorong estuary is not yet possible due to a superficial understanding of the species population dynamics, it is clear that freshwater inflows have a positive effect on population size.

The findings of the present study suggest that the Coorong estuary is likely to play an important role in the population dynamics of *R. tapirina*. The distribution and abundance patterns of *R. tapirina* within the system were highly variable, fluctuating in time and place. It is likely that these patterns were determined partly by variation in the magnitude of freshwater inflow to the system because large areas of high quality, sheltered and food-rich habitat that support high abundances of *R. tapirina* are only available during and immediately after years of increased freshwater inflow. Furthermore, the periodic nature of the variation in population abundance suggests that it is likely that the level of fishing mortality experienced in the peak fishing years also had some impact on the population dynamics of the species. In the next four chapters, I explore the demographic processes that may contribute to these patterns in population abundance and examine the effect of fishing on the demography of the population.

3   Age validation, growth and population characteristics of greenback flounder (*Rhombosolea tapirina*) in a large temperate estuary

3.1   Introduction

Fish population dynamics are driven by a range of demographic processes that are most effectively measured using fish age as a time reference (Campana 2001). The most convenient and effective method for estimating fish age is through the direct interpretation of hard, calcified anatomical structures that demonstrate an incremental structure (Beckman and Wilson 1995; Campana 2001). Fish otoliths have proven to be the most effective structures for this purpose (Campana and Thorrold 2001). An otolith grows through the regular deposition of new material to its growing surface, forming an optical pattern that reflects seasonal metabolic processes of the fish which is not subsequently resorbed or reworked (Campana 1999). Whilst otolith validation studies for a broad range of taxa from different environments have demonstrated annual periodicity of increment formation (Choat et al. 2009), the method of otolith examination can influence the accuracy of the interpretation and the resulting estimate of age (Fowler 2009). Furthermore, since the clarity of increments varies amongst taxa and because otolith interpretation is a skill that is subject to human error (Morison et al. 2005), it is recommended that considerable effort be spent in establishing a fish ageing protocol, particularly when the target species is vulnerable to over-exploitation by fishing (Choat et al. 2009).

For a fish species to be suitable for otolith-based age determination, its otoliths must fulfil three criteria (Fowler 1990): (1) they must display an incremental structure that can be interpreted visually; (2) growth increments must be formed on a regular, determinable time scale; and (3) otoliths must continue to grow throughout the lives of the fish. Prior to the 1980s, most ageing studies were based on interpreting the structure of whole otoliths (Beamish 1992). Since then, thin sections of otoliths have been used because differentiating closely spaced increments near the outer edge of whole otoliths can be difficult due to the way otoliths grow (Mann-Lang and Buxton 1996; Fowler 2009). Nonetheless, studies for some species that have compared the usefulness of both methods indicated that whole otoliths provided reliable age estimates (Williams et al. 2003, 2005; Garcia-Mederos et al. 2010). As such, determining the most appropriate ageing protocol for

a particular species requires undertaking a comparison of the interpretability of whole and sectioned otoliths.

Validated age and growth information for temperate flatfish is generally limited to species in the northern hemisphere (Nash and Geffen 2005). The greenback flounder, *Rhombosolea tapirina*, is the most common pleuronectid in Australian waters (van den Enden et al. 2000). This species is distributed in estuaries and coastal waters to depths of 100 m along the southern coast of Australia and in New Zealand (Gomon et al. 2008), where it supports commercial and recreational fisheries (Kailola et al. 1993; Froese and Pauly 2013). In South Australia, almost all catches are taken from the Coorong estuary (Ferguson 2007). This large estuarine system lies at the terminus of Australia's largest river system, the Murray-Darling. Long-term statistics for this fishery indicate extreme inter-annual and spatial variation in population biomass and abundance (Ferguson 2007). The processes driving this variation remain poorly understood due to a superficial understanding of the population dynamics of the species.

Information on the population dynamics of *R. tapirina* is limited. In south-eastern Australia, *R. tapirina* attain sexual maturity at approximately 203 mm total length (Ye et al. 2013), whilst reproduction occurs during the cooler months from March to October in both estuaries and offshore environments (Kurth 1957; Crawford 1984; Barnett and Pankhurst 1999). Post-larval recruits settle out into shallow, unvegetated sandy habitats between July and October (May and Jenkins 1992). However, there is no understanding of the demographic processes that operate beyond these early life stages. Whilst unvalidated estimates of age, based on counts of opaque zones in the otoliths of adult *R. tapirina,* suggested a maximum age of 10 years, several nearshore populations in New Zealand and Tasmania consisted mostly of fish less than 3 years of age (Kurth 1957; Sutton et al. 2010). There is uncertainty about these age estimates because of the lack of a validated ageing protocol. The development of such a protocol and providing estimates of age and growth for *R. tapirina* are essential to better understand the population dynamics and demography of the species.

The present study addressed the lack of demographic information for *R. tapirina* by providing an assessment of the usefulness of its otoliths for ageing and estimates of age-based data for an exploited population in the Coorong estuary in South Australia. The specific objectives addressed were: (1) to assess the usefulness of whole and transverse sections of the otoliths of *R. tapirina* for ageing by determining whether they fulfil the three criteria described above, and to develop an

algorithm for age determination in months; (2) to compare growth between male and female fish, and to determine whether growth varies spatially; and (3) to describe the population characteristics in terms of size and age structures from two areas in the Coorong estuary, and determine how these structures varied over a three-year period.

3.2   Materials and methods

3.2.1   Study area

This study was done in the Coorong estuary, South Australia (Figure 3.1). This estuarine system is a long (~110 km), narrow lagoon that extends south-east from the river mouth and represents one of the largest estuarine habitats in Australia. Due to drought conditions, freshwater inflows to the system were minimal from 2002 to 2010, with no flows between 2007 and 2009. The lack of flows contributed to an extensive longitudinal salinity gradient from the mouth of the estuary (Webster 2010). Typically, salinity ranged from marine (30 – 36 g L^{-1}) near the mouth, to hypersaline (>120 g L^{-1}) in the southern parts of the Coorong (Kingsford et al. 2011). In late 2010, freshwater inflows recommenced and continued throughout 2011. These flows reduced salinity to mostly fresh (< 4 g L^{-1}) in the vicinity of the mouth, while hypersalinity (> 90 g L^{-1}) persisted in the southern Coorong. The estuarine system supports shallow ($\leq$ 8 m), sandy habitats, with soft-muddy sediments in deeper areas (Gilbertson 1981). A multi-species, commercial fishery operates throughout the system and targets several finfish species, including *R. tapirina*.

3.2.2   Sample collection and processing

In total, 851 *Rhombosolea tapirina* were collected between December 2008 and November 2011 from two study areas. One study area, the Estuary, was located near the Murray mouth and included all waters downstream of the Goolwa Barrage to the Ewe Island Barrage (Figure 3.1). The other, North Coorong, extended from the Ewe Island Barrage to Long Point. Fish were collected from the commercial fishing sector which targets *R. tapirina* with 4.75″ mesh gill nets. Such nets are size-selective and rarely capture fish < 220 mm and > 360 mm total length (TL). Scientific sampling was also done using 3.5″ mesh haul nets to capture a broader size range of fish, i.e. >150 mm TL. Sampling effort and catch rates varied throughout the study period (Table 3.1), but fish were collected in most months. For the Estuary, fish were sampled regularly between December 2008 and May 2011, while for North Coorong sampling occurred between June 2009 and November 2011 (Table 3.1).
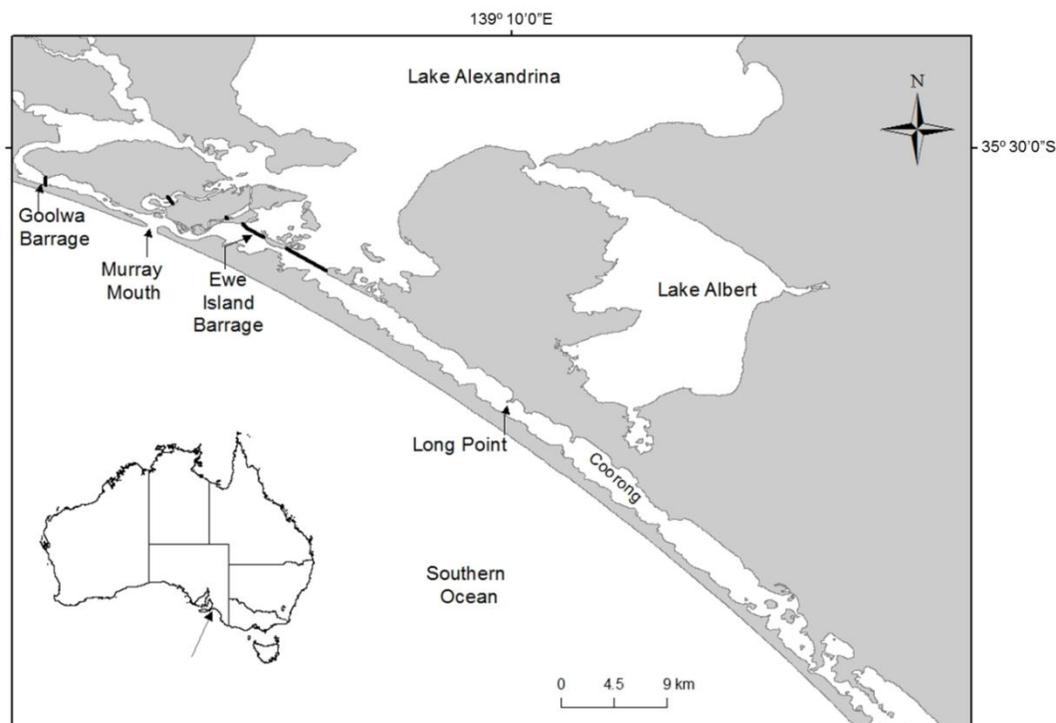
Figure 3.1 Map of the Coorong estuary, showing the two study areas: the Estuary (from Goolwa Barrage to the Ewe Island Barrage) and North Coorong (from Ewe Island Barrage to Long Point). Inset shows location of study region relative to Australia. The locations of the barrages that separate the estuarine system from Lake Alexandrina are indicated by black lines.

Table 3.1 Summary of the characteristics of the samples of *R. tapirina* from the Estuary and North Coorong that were considered for age determination and for analysis of population characteristics (n = 851).

Area	Sampling period	Sample size (n)	Size range (mm)	Mean TL (±SD) (mm)
Estuary	Dec 08 – May 09	104	162 - 359	276 (±56)
	Jun 09– Nov 09	124	157 - 385	286 (±34)
	Dec 09 – May 10	142	171 - 336	262 (±46)
	Jun 10– Nov 10	177	185 - 350	247 (±28)
	Dec 10 – May 11	22	231 - 313	267 (±21)
North Coorong	Jun 09 – Nov 09	24	271 - 357	314 (±26)
	Dec 09 – May 10	127	186 - 327	271 (±33)
	Jun 10 – Nov 10	77	157 - 321	228 (±45)
	Dec 10 – May 11	24	154 - 331	225 (±41)
	Jun 11– Nov 11	30	291 - 336	307 (±10)

Most fish were processed within eight hours of capture. Each fish was measured for TL to the nearest mm and weighed to the nearest 0.01 g. The gonads were examined to determine the sex of the fish. The sagittae, i.e. the largest pair of otoliths, were removed, cleaned, dried and stored. Later, the left otolith from each fish was prepared for ageing. It was embedded in polyester resin and then a transverse section (TS) of approximately 400 μm thickness that incorporated the core was removed using a low speed diamond saw (Struers Accutom) (Fowler and McGlennon 2011). The section was glued to a labelled glass microscope slide.

3.2.3   *Assessing the usefulness of the otoliths for ageing*

Internal structure of otoliths

To determine the presence and interpretability of incremental structure in the otoliths and to develop a protocol for ageing this species (criterion 1), whole and TS of otoliths from the same fish were examined independently. The TS of each otolith was smeared with immersion oil (Scharlau ©) and examined under transmitted light using a dissecting microscope. The opaque zones surrounding the core were counted. On a different day, the whole right otoliths from the same fish were immersed in water, with the distal surface upwards, and examined with a dissecting microscope using reflected light, and the opaque zones were counted. Counts from whole and sectioned otoliths were compared to assess the interpretability of the otolith structure using both methods and to identify the preferred method for ageing determination. Counts were compared by constructing an age-bias plot, whereby the mean count from whole otoliths was plotted for groups of otoliths that contained the same number of opaque zones in the TS. Counts were also compared by calculating the average percent error (APE) for each technique (Beamish and Fournier 1981). Low values of APE indicated higher precision. Otoliths that did not display an interpretable structure were not used for comparison, or later for age determination. Overall, the otolith pairs from 627 fish were examined, of which 21 (3.4 %) were rejected as unreadable.

Timing of opaque zone formation

To determine if the structure in the otoliths of *R. tapirina* formed on a regular, determinable time scale (criterion 2), the timing of formation of opaque zones was investigated. In both 2009 and 2010, young-of-the-year *R. tapirina* were collected monthly. Their otoliths were examined to determine when the first opaque zone formed, using edge analysis. The TS of these otoliths were examined with a dissecting microscope using transmitted light to differentiate those otoliths with: (1) a translucent edge; (2) an opaque edge; and (3) a completed opaque zone with recently formed

translucent material on the edge. The percentage occurrence for each edge type was calculated for each month.

For the otoliths of older *R. tapirina*, marginal increment analysis was done. Where possible, fish were collected in each month between January 2009 and January 2011 and were pooled across the two study areas. Marginal increment analysis could only be applied to formation of the second opaque zone, as older fish were not sampled in sufficient numbers. Each otolith was examined with a dissecting microscope using transmitted light, and the distances between the outer edge of the opaque core and the outer edge of the first opaque zone, and then to the edge of the otolith, were measured toward the proximal surface along the ventral side of the sulcal groove (Figure 3.2). The marginal increment for each otolith was expressed as a proportion of the immediately preceding annulus and these were plotted for each month of the year. The appearance of the otolith margin was recorded as either opaque or translucent. All otoliths were read without reference to fish size and the time of year the fish was captured.

Growth of otoliths

To determine if otoliths grew continually throughout the lives of the fish (criterion 3), two measures of otolith size were recorded and related to the number of opaque zones. Whole otoliths from 280 fish were weighed to the nearest 0.01 mg and then sectioned. For each TS, the otolith thickness, i.e. the distance from the centre of the otolith core to the proximal surface, was measured (Figure 3.2). The estimates of otolith size were compared to the number of opaque zones in the TS using linear regression. The statistical program SPSS V. 19 was used for these statistical procedures.

Development of an ageing protocol

To provide an estimate of age in months for each fish, an algorithm was developed that considered the periodicity of opaque zone formation, the date of capture, and the universal birthdate of 1 June, i.e. the middle of the reproductive season (Crawford 1984; Barnett and Pankhurst 1999). The algorithm was based on the results of edge analysis and marginal increment analysis which identified the timing of formation of the first and second opaque zones.

3.2.4   Growth

Estimates of length-at-age for male and female *R. tapirina* were used to describe growth using von Bertalanffy growth curves: $L_t = L_\infty [1 - e^{-K(t-t0)}]$, where L_t = length at age, L_∞ = theoretical

maximum or asymptotic length that fish would reach if they lived indefinitely, K = curvature parameter, and t_0 = the theoretical age at zero length (von Bertalanffy 1938; Beverton and Holt 1957). The growth parameters were estimated using a non-linear least squares von Bertalanffy growth model in the statistical package R (R Development Core Team, 2011). The parameter t_0 was constrained to zero to provide a better description of early growth (Williams et al. 2003). Univariate analysis of variance (ANOVA) was used to perform step-wise maximum likelihood ratio tests to statistically compare growth parameters K and L_∞ between males and females (Kimura 1980, Francis 1988b). This procedure also allowed growth relationships to be compared graphically using estimates of 95 % confidence ellipses generated around least squares estimates of K and L_∞ (Kimura 1980). For the spatial comparison, growth curves were compared between the two study areas using size-at-age data that were pooled across sexes.

3.2.5   *Population characteristics*

Estimates of size-at-age of fish collected from the two study areas were used to produce area-specific population size and age structures for each 6-month period of the study. The sex ratios of the populations in both areas for each sampling period were also determined. A chi-square test for goodness-of-fit was used to assess whether the relative frequency of males and females for each 6-month period conformed to a 1:1 ratio.

3.3   Results

3.3.1   *Usefulness of otoliths for ageing*

Interpretability of otolith structure

Whole otoliths contained an opaque core that was surrounded by a translucent zone of variable thickness (Figure 3.2). Beyond this, larger otoliths displayed alternating opaque and translucent zones which were often difficult to distinguish towards the edge. TS of otoliths were essentially translucent with relatively distinct opaque zones. In TS of larger otoliths, an alternating sequence of opaque and translucent zones extended toward the dorsal and ventral edges and proximal surface. Zones were most pronounced along the ventral axis from the core to the proximal surface.
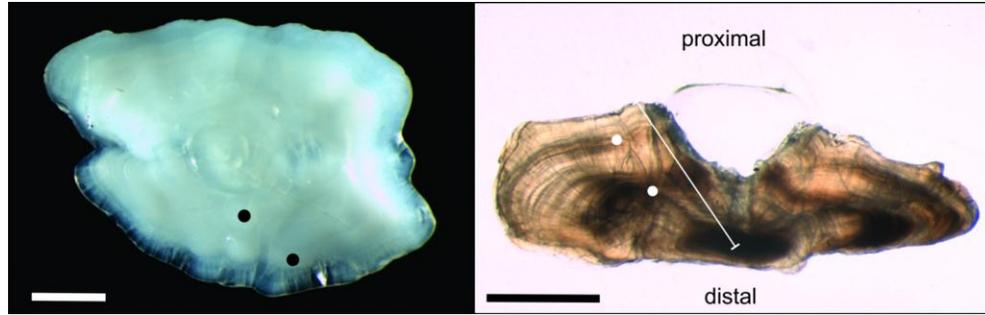
Figure 3.2 Comparison of a whole otolith (left) and TS (right) of an otolith of *R. tapirina* with two opaque zones. Whole otolith illuminated with reflected light with the distal surface facing upwards. Black dots indicate opaque zones that were interpreted as annuli. TS of the corresponding otolith from the same fish illuminated with transmitted light. While capped line indicates the axis along which measurements were made for marginal increment analyses and otolith thickness. White dots indicate the opaque zones that were interpreted as annuli. Scale bars = 1 mm.

In order to compare the interpretability of their incremental structures, counts of opaque zones were compared between whole and sectioned otoliths from the same fish (Figure 3.3). Many otoliths displayed between one and three opaque zones, whilst for a large proportion, no opaque zones were evident. For 91 % of the otolith pairs, counts were the same between whole and sectioned otoliths, suggesting that features were generally consistent between the pair. However, for otoliths with two or three opaque zones, the count from the whole otolith was often lower than for the TS (Figure 3.3). The APE was 2.3% for otoliths with no opaque zones, which increased to 5.7%, 10.8% and 13.5 % for those with one, two and three zones, respectively. This indicates that the incremental structure of the whole otoliths became more difficult to interpret accurately as they got larger, resulting in counts from such otoliths underestimating the true count. As such, the use of TS was determined as the preferred method for age determination.
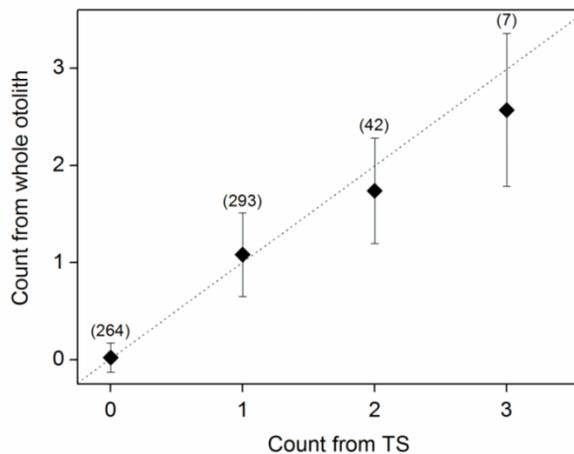
Figure 3.3 Age-bias plot comparing the mean count of opaque zones from whole otoliths (±SD) to the counts from the TS of otoliths, for groups of otoliths that contained the same number of opaque zones in the TS. Sample sizes for each group are shown in brackets.

<u>Timing of opaque zone formation</u>

TS of otoliths from young-of-the-year *R. tapirina* from 2009 and 2010 were examined to
determine when the first opaque zone was formed. In both years, the otoliths from fish collected
between March and July had translucent edges whilst otoliths with opaque edges were found from
August–December (Figure 3.4). Otoliths with a completed opaque zone and a translucent edge
were first observed in September, and increased in frequency until all otoliths were like this by
January. These results indicated that the formation of the first opaque zone was initiated in mid-
to-late winter in the fish's second year and was completed by mid-summer.
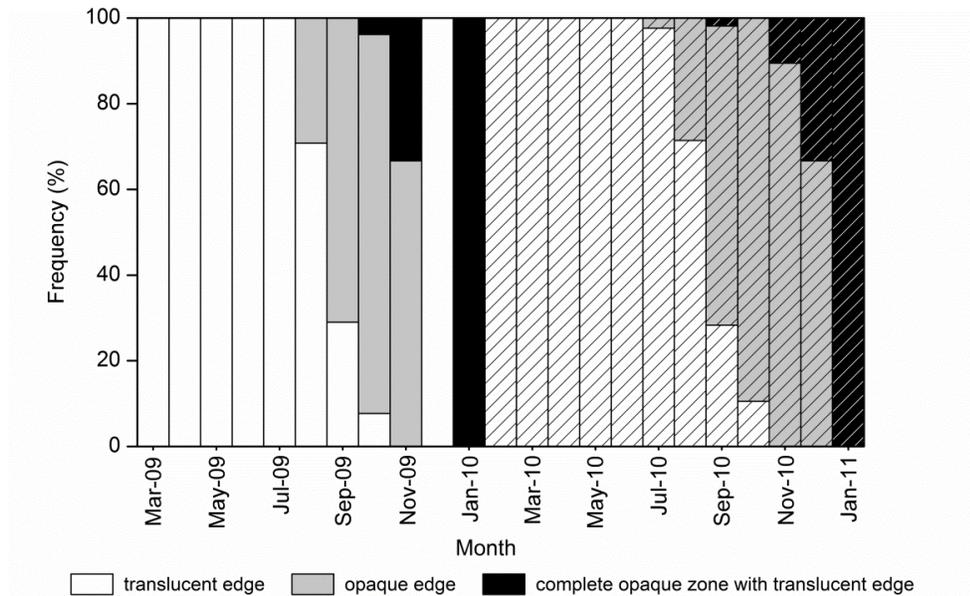
Figure 3.4 Monthly time series showing the results of edge analysis from TS of otoliths from young-of-the-
year *R. tapirina* collected in 2009 (non-hatched bars) and 2010 (hatched bars) (*n* = 486).

Those fish with one complete opaque zone were considered for marginal increment analysis. In
2009 and 2010, all otoliths collected between January and June had a translucent edge, with the
marginal increment increasing through this period (Figure 3.5). Some collected in July and most
from August to November had opaque margins, indicating that the formation of the second
opaque zone occurred from July onwards in both years. By December, otoliths had translucent
margins and the formation of the new opaque zone had been completed (Figure 3.5). These results
from marginal increment and edge analysis over a 2-year period were consistent with the annual
deposition of a single opaque zone during late winter which was completed by early summer.

Growth of otoliths

The size of otoliths increased with the number of opaque zones in the TS for both measures considered. The two measures of otolith size, otolith weight (OW = 2.318*no. of opaque zones + 5.097; P < 0.001; n = 279) and otolith thickness (OT = 2.214*no. of opaque zones + 0.8606; P < 0.001; n = 279) increased proportionally with the number of opaque zones counted in the TS and these linear relationships accounted for 53.2 % and 54.7 % of the variation in otolith size, respectively. These results indicate that the otoliths continued to grow throughout an individual's life. As such, their incremental structure should reflect their age.
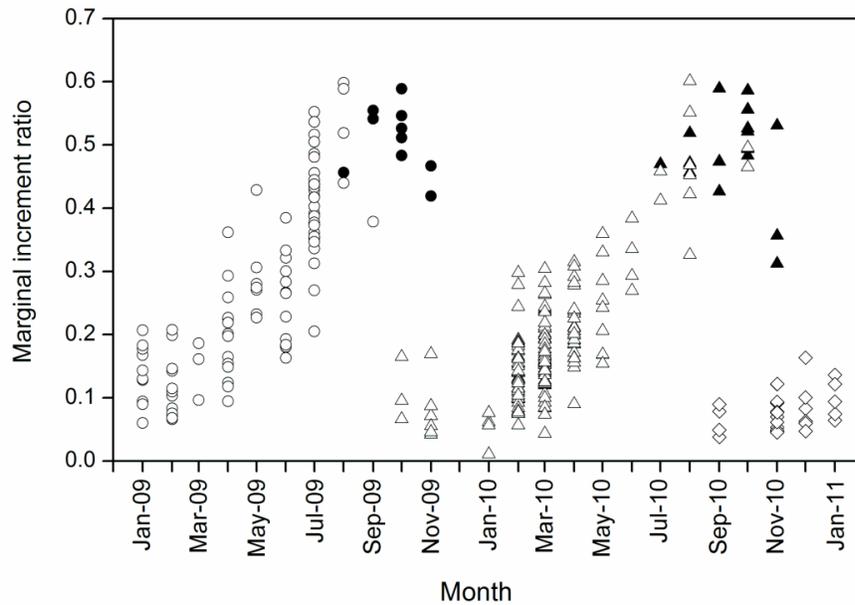
Figure 3.5 Monthly time series showing the marginal increment ratio (proportion of the immediately preceding annulus) for TS of otoliths from *R. tapirina* that displayed one fully formed opaque zone, i.e. 1+ age class, for the 2007 (circle), 2008 (triangle) and 2009 (diamond) year classes (*n* = 346). The edge type, e.g. translucent edge (open symbols) or opaque edge (closed symbols) is also shown.

Development of an ageing algorithm

A protocol was developed to provide age estimates for *R. tapirina* from the interpreted otolith structure. The algorithm was: $A_m = (N \times 12) + m_b + m_c$, where A_m = age in months, N = number of opaque zones, m_b = number of months from the birth date to the end of the year (7 months for *R. tapirina*, i.e. June – December), and m_c = number of months from the start of year to the month of capture.

3.3.2   Growth

Von Bertalanffy growth curves were fitted to the size-at-age data for males and females to compare growth between sexes for the two study areas. Maximum likelihood tests indicated no significant difference between sexes for the estimates of K and L_∞ for the Estuary ($F_{1,552}$ = 0.002, p = 0.965) and North Coorong ($F_{1,272}$ = 0.062, p = 0.804) (Figure 3.6A,B). As such, data were pooled across the sexes to examine the spatial variation. The maximum likelihood test indicated that L_∞ was marginally higher for the Estuary (L_∞ = 348 mm TL) compared to North Coorong (L_∞ = 340 mm TL) ($F_{1,831}$ = 0.002, p = 0.014), though differences were relatively small (Figure 3.6C). The growth parameter K was the same for each area (K = 0.09) ($F_{1,830}$ = 1.1097, p = 0.293). Spatial differences in growth were supported by non-overlapping 95% confidence ellipses around the estimates of K and L_∞ (Figure 3.6C). Consequently, there were slight differences in size-at-age of *R. tapirina* between areas. On average, fish grew to 231 mm TL and 220 mm TL in the Estuary and North Coorong, respectively, in their first 12 months of life. From then, growth rate decreased, but was similar in both areas, with size gains of 77 mm, 27 mm and 8 mm in their second, third and fourth years, respectively.
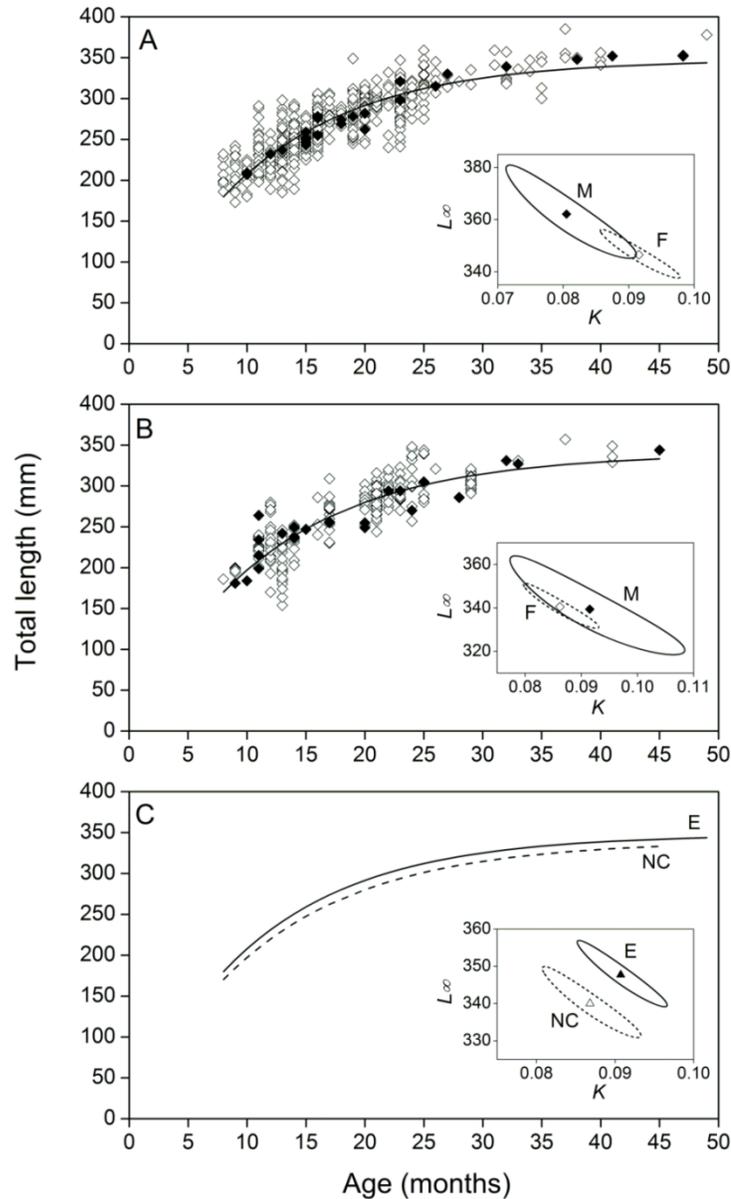
Figure 3.6 Size-at-age data for female (open diamonds) and male (closed diamonds) *R. tapirina* from A the Estuary and B North Coorong with fitted von Bertalanffy growth curves. C, site-specific growth curves are shown for the spatial comparison whereby data were pooled across sexes for the Estuary (E) and North Coorong (NC). Also displayed are 95 % confidence ellipses for *K* and *L∞* for each sex from the two study areas, and for each area.

3.3.3   *Population characteristics*

Size and age structures

For the Estuary, there were considerable differences in the size structures across consecutive six-month periods (Figure 3.7). Fish ranged in size from 157 to 385 mm TL. Size distributions generally consisted of a single, dominant mode. One exception was the bimodal distribution for

the period of December 2009 – May 2010 that involved a small mode at 210 mm TL and a large mode at 310 mm TL. The small mode persisted through late 2010 and into 2011. However, the mode of larger fish was no longer evident in the size structures in subsequent periods, with fish greater than 330 mm TL being rare in 2010 and 2011.

The six-monthly age distributions for the Estuary involved only a small number of age classes and were generally dominated by the 1+ age class (Figure 3.7). For the period of December 2008 - May 2009, this age class accounted for 55 % of the sample, with 0+, 2+ 3+ fish accounting for 30 %, 14 % and 1 %, respectively. In 2010 and 2011, older fish were rare, with the 1+ age class accounting for >83 % of fish in the two six-month age distributions from June 2010 to May 2011. In both years, the contribution of 0+ fish was highest from December – May. For each six month period females accounted for 85-99 % of the fish sampled. On each occasion, the Chi-square goodness-of-fit tests indicated a significant difference in sex ratio at the 0.0001 level of significance.

For North Coorong, there were substantial differences in size distributions among six-month periods. Fish ranged in size from 154 – 357 mm TL (Figure 3.8). Gill net sampling limited the size range of fish collected in June - November 2009, thus there was a narrow size distribution around the mode of 310 mm TL. In early 2010, these large fish began to drop out of the size structures, and by mid-2010 were absent with very few fish >280 mm TL. As a result, small fish dominated the size distribution in late 2010. This cohort persisted in subsequent size structures and contributed to a dominant mode of larger fish one year later.

Overall, age distributions for North Coorong involved four age classes, i.e. 0+ to 3+, but were dominated by either 1+ or 2+ fish (Figure 3.8). In 2009, 58 % were 2+ fish, while most of the remainder were 1+. In 2010, the age structures were dominated by 1+ fish, whilst 2+ fish were present in relatively low numbers. The high proportion of 1+ fish at that time was no longer evident as 2+ fish the next year. In contrast, the high proportion of 1+ fish in the population in early 2011 was still evident as 2+ later that year. In each year, the contribution of 0+ fish to the adult population was highest from December – May. Females accounted for between 93 – 100 % of the fish sampled in each six-month period. On each occasion, Chi-square goodness-of-fit tests indicated a strong bias in sex ratio at the 0.0001 level of significance.
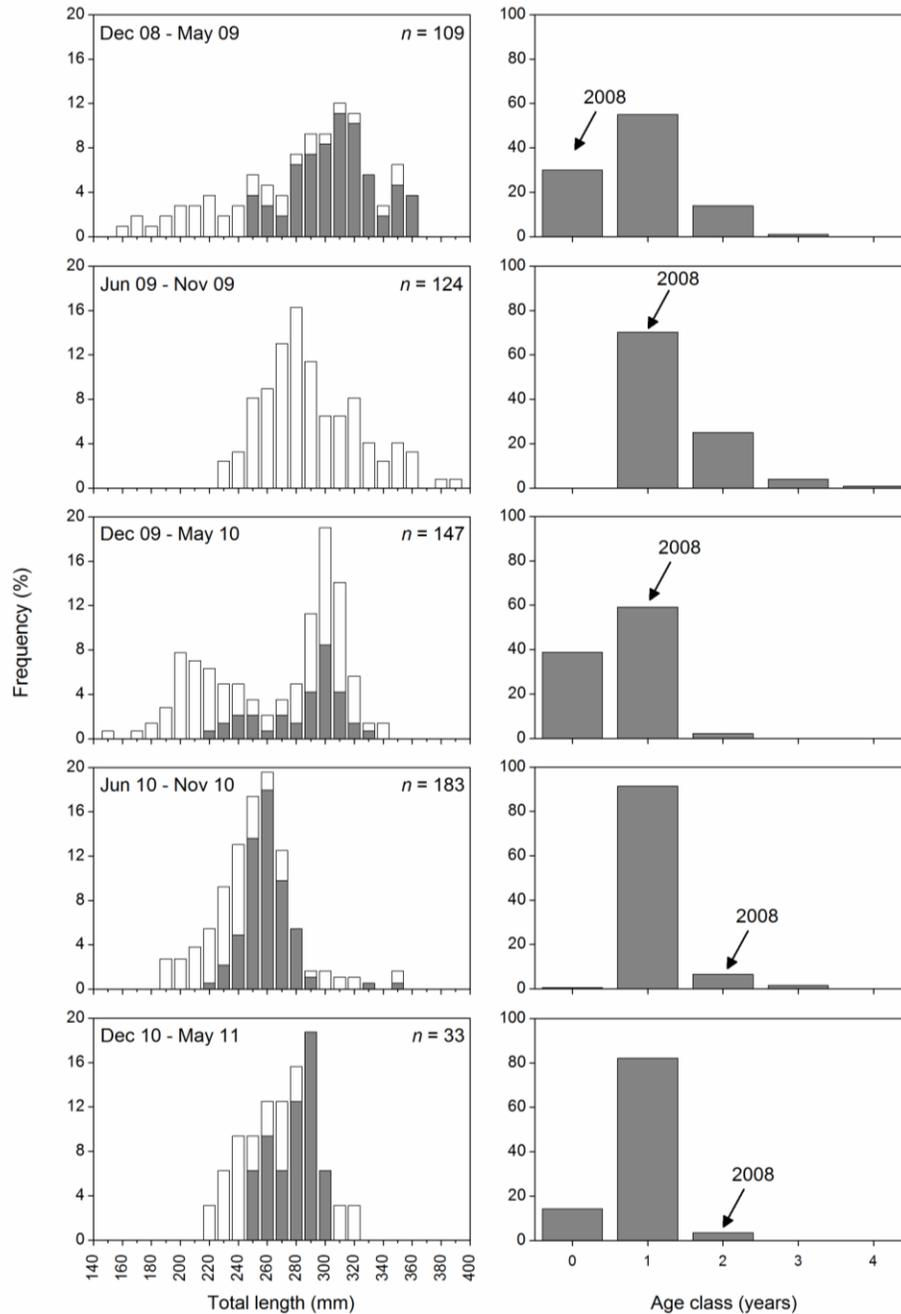
Figure 3.7 Size and age structures of *R. tapirina* from the Estuary for the period December 2008 – May 2011. Left-hand graphs show the size structures in consecutive 6-month periods. Grey bars represent samples collected from the commercial fishery and white bars represent samples collected by scientific sampling. Right-hand graphs show the age structures for the same consecutive periods. The progression of the 2008 year class is also shown.
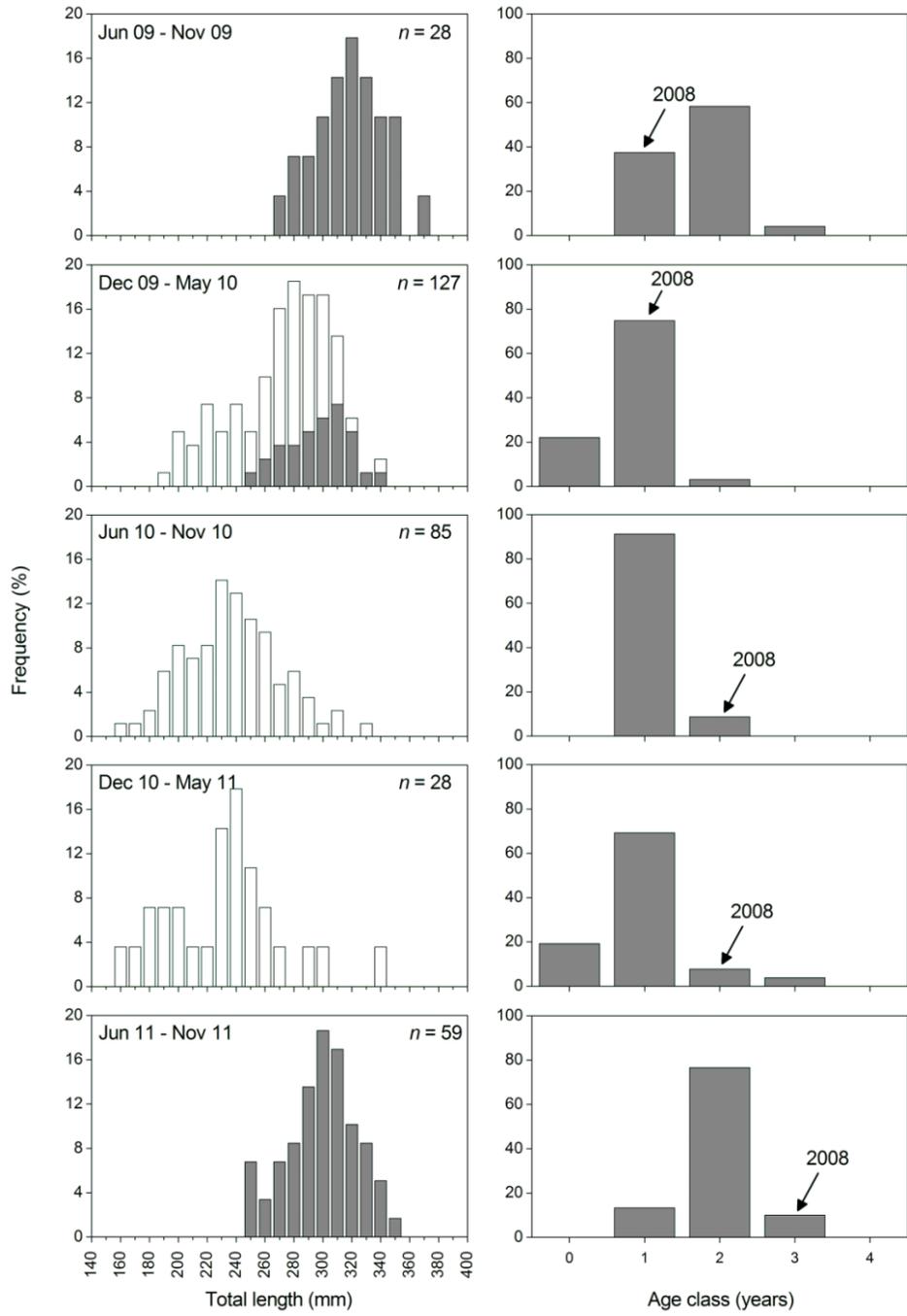
Figure 3.8 Size and age structures of *R. tapirina* from North Coorong for the period June 2009 – November 2011. Left-hand graphs show the size structures in consecutive 6-month periods. Grey bars represent samples collected from the commercial fishery and white bars represent samples collected by scientific sampling. Right-hand graphs show the age structures for the same consecutive periods. The progression of the 2008 year class is also shown.

3.4   Discussion

3.4.1   *Usefulness of the otoliths for ageing*

The first criterion for otoliths of *R. tapirina* to be useful for ageing is that they must display an internal structure that can be interpreted visually. Here, whole and TS of otoliths displayed an incremental structure that consisted of alternating opaque and translucent zones. This macro-structure was clearest in the TS. The use of whole otoliths for age determination remains a common practise for the youngest age classes of some flatfish species (Dwyer et al. 2003). Here, whole otoliths from larger fish that contained two or more opaque zones were thick, which restricted their transmittance of light, obstructed the view of their internal structure, and complicated their interpretation. This resulted in counts of opaque zones from whole otoliths that were often lower than those from the sectioned otolith from the same fish. Therefore, the preferred method to apply in an ageing protocol for *R. tapirina* was to prepare TS of otoliths in order to most clearly reveal the incremental structure and thereby ensure the highest accuracy in age determination.

The second criterion for the usefulness of the otoliths of *R. tapirina* is that the incremental structure must be formed on a regular and determinable time scale. In this study, edge analysis determined that the timing of formation of the first opaque zone was between July/August and December, i.e. late winter – summer, during the fish's second year of life. Completion of the first opaque zone by December corresponded to an age of 19 months. Furthermore, edge analysis and marginal increment analysis indicated that the second opaque zone was formed between July and December one year after the first zone was formed. Therefore, these results substantiate an annual periodicity to the formation of the otolith macrostructure through the first 2-3 years of the lives of the fish.

While the periodicity of formation of opaque zones subsequent to the second one remains unvalidated for now, there is evidence that the third zone is also formed annually. First, it is similar in appearance to the second zone. Secondly, the deposition of translucent and opaque material that comprised the third increment conforms to a similar temporal cycle to that of the previous zone. Verification of the periodicity of formation across the entire age range of interest is the preferred outcome of any validation study, however as Campana (2001) advised, this is often unrealistic. At a minimum, validation for the youngest age classes is imperative (Choat et al. 2009).

The timing of zone formation described here for *R. tapirina* is characteristic of numerous temperate and sub-tropical marine fishes (Beckman and Wilson 1995; Fowler 2009). For some species the formation of opaque zones coincides with periods of rapid growth (Beckman and Wilson 1995; Potts and Cowley 2005). This is unlikely for *R. tapirina* as water temperatures across southern Australia during spring are relatively low. Furthermore, this is the spawning season for *R. tapirina* (Crawford 1984; Barnett and Pankhurst 1999), during which higher demands for energy for gonad development are likely to limit growth (Rijnsdorp 1990; Nash and Geffen 2005). As such, opaque zones in the otoliths are likely to correspond to slow otolith growth and to narrower widths of daily increments (Fowler 2009; Mann-Lang and Buxton 1996). Nonetheless, the process of opaque zone formation is complex and likely involves numerous exogenous (Fowler and Doherty 1992), endogenous and physiological (Fowler 1995) factors.

Finally, the third criterion for otoliths to be useful is that they must continually grow throughout the lives of the fish. For *R. tapirina,* otolith size increased proportionately through the first four years of life. This suggests that relatively consistent amounts of otolith material were deposited on the growing surface of the otoliths during each year of the first four years of the lives of the fish.

In summary, this study has demonstrated that the incremental structure in the TS of *R. tapirina* otoliths is formed annually and therefore can be interpreted in terms of fish age. As such, an ageing protocol was developed to convert counts of opaque zones in the TS of otoliths into estimates of fish age. To develop an informative growth model for fast-growing, short-lived species such as *R. tapirina*, it was advantageous to reduce the temporal resolution of size-at-age estimates from years to months.

3.4.2   Growth

Estimates of size and age in months were used to describe growth for *R. tapirina*. Since the growth rate decreased exponentially with age, von Bertalanffy growth curves were used to describe and compare growth between sexes. For the Estuary and North Coorong, there were no differences in growth parameters between males and females. For many flatfish species, sexual dimorphism in growth is characteristic, with females typically attaining larger sizes and living longer than males (Dwyer et al. 2003; Fischer and Thompson 2004; Nash and Geffen 2005). However, for *R. tapirina,* there were no such differences detectable for the first 4 years of life. This may relate, in part, to the few male fish and the young age classes available to this study.

Such gender-based differences, however, could possibly become evident in older fish for which energy has been diverted to reproduction for a longer part of their lives.

Growth parameters were also compared between the Estuary and North Coorong. Fish in the Estuary attained a higher asymptotic length than those from North Coorong. However, the area-specific growth curves indicate that the spatial difference for L_∞ was small (8 mm TL) in relation to the estimated maximum size and life span of the species. The non-explicit spatial difference suggests that either environmental conditions, including the availability of resources for *R. tapirina*, were similar among areas, and/or there was some exchange of individuals between the two areas. Nonetheless, area-specific growth curves indicated that *R. tapirina* achieved approximately 66 % (231 mm TL) and 64 % (220 mm TL) of their respective L_∞ after 12 months of life in the Estuary and North Coorong, respectively. Whilst rapid growth is characteristic of the genus *Rhombosolea*, the growth of *R. tapirina* in its first year is much greater than for its conspecifics (Coleman 1974; Francis 1988a; Paul 1992).

3.4.3   Population characteristics

Size and age characteristics of *R. tapirina* were examined to inform about population structure and demographic processes likely to account for fluctuations in population size. From December 2008 – November 2011, size structures were comprised mainly of fish less than 340 mm TL and the age structures were generally dominated by 1+ and 2+ fish, while older fish were rare. Given the potential for this species to attain 10+ years of age (Sutton et al. 2010), the biomass of *R. tapirina* in the Coorong estuary essentially consisted of fish in the youngest age classes.

The dominance of only one or two young age classes observed here is similar to other populations of *R. tapirina* in Tasmania and New Zealand. Kurth (1957) indicated that fished populations off the north coast of Tasmania consisted mainly of 1+ and 2+ fish. Similarly, exploited inshore populations off the south coast of New Zealand mainly involved 2+ and 3+ fish (Sutton et al. 2010). However, the latter population also included fish that were up to 10 years of age, thus demonstrating the potential for this species to achieve considerably older ages than were found in the present study. As such, the population in the Coorong estuary appears to be considerably truncated in age, as there is minimal biomass stored in the age classes of 3 years of age and older.

There are a number of potential hypotheses to account for the observed population truncation. Exploited fish populations typically experience some degree of age truncation through the

removal of larger, older fish by size-selective fishing (Longhurst 1998). For *R. tapirina*, historically low exploitation rates by the commercial fishery in the years prior to (Ferguson 2007), and during this study suggest that other processes contributed to the restricted age structure of the population. Another hypothesis is that the older fish migrate from the estuary to offshore habitats, possibly for spawning. Such behaviour occurs for numerous other flatfish species such as *Pleuronectes platessa* and *Atheresthes stomias* (Rijnsdorp et al. 1990; Rickey 1995). Perhaps during the drought, the poor environmental conditions in the Coorong estuary forced the adults to leave the estuary for the coastal environment due to a lack of food or appropriate conditions for reproduction. As yet, it is not possible to differentiate amongst these alterative hypotheses to account for the truncated populations.

A further complication for the population characteristics of this species was the change in age structure that followed the commencement of drought-breaking freshwater flows to the estuary in late 2010. Flows rapidly reduced salinity throughout most of the system and improved habitat quality for *R. tapirina*. As a consequence, the 1+ fish which dominated the population in North Coorong during the final year of the drought period remained in the population and contributed a rare dominant mode of 2+ fish in 2011. One hypothesis is that the retention of these fish in the system for a further year occurred in response to increased food availability (Grange et al. 2000) and the re-opening of suitable habitat for *R. tapirina* in the Coorong. The question remains as to the influence of environmental variation associated with freshwater flows on demographic processes that may contribute to fluctuations in population size and structure.

Despite the age truncation, there were slight variations in population structure over the three-year period. The size and age structures were relatively similar between six-month periods for both study areas, due to a lack of modal progression of 1+ fish across years. Recruitment of 0+ fish occurred in summer/autumn of each year and older individuals in their third or fourth years of life were rarely detected at that time. Moreover, there were strong spatial similarities in population structure between the Estuary and North Coorong, which suggests that either mixing occurred between the two areas, and/or that similar demographic processes were operating over this spatial scale.

This study has established the usefulness of sectioned otoliths for ageing *R. tapirina* and has contributed important information on the population biology of this species. The truncated nature of the populations in the Coorong estuary indicate that the previously-documented variation in

fishery productivity for this species is likely driven by demographic events and/or processes that occur during the first two years of the life history. This is likely to reflect inter-annual variation in recruitment or variable post-settlement survivorship in this environmentally-complex system.

4   Reproductive characteristics and spawning dynamics of greenback flounder (*Rhombosolea tapirina*) in a large temperate estuary under drought conditions

4.1   Introduction

Understanding the reproductive dynamics of fish is of fundamental importance to the management of exploited fish populations. Fish reproduction is complex and involves numerous physiological processes that are regulated in part by the conditions of the environments they occupy (Pankhurst and Munday 2011). Differences in the cellular processes of egg production and development, as well as the behavioural aspects of spawning, characterise the reproductive strategies adopted by different fish species to enhance the likelihood of reproductive success (Fowler et al. 1999). As such, knowledge of the reproductive biology of individual species and how it is influenced by the environment will contribute to understanding their population dynamics.

Fish species that occupy temperate environments commonly display seasonality in their reproductive development that aligns with specific suites of environmental variables (Pankhurst and Porter 2003). While photoperiod and temperature are commonly viewed as the primary environmental determinants of reproductive development for temperate fish, other factors including salinity and food availability can also have a strong influence, particularly for estuarine species (Leblanc et al. 1997; Sumpter 1997). As such, the reproductive dynamics of estuarine species are often quite plastic, displaying considerable spatial and temporal variation in the timing and duration of gonad development, frequency of spawning events and fecundity (Duarte and Alcaraz 1989; Potter et al. 1990; Sarre and Potter 1999). In extreme cases, fish may skip spawning completely and forego egg production until the subsequent year in response to unfavourable conditions (Rideout and Tomkiewicz 2011). Such plasticity highlights the need to understand seasonal variation of the reproductive biology of individual species in order to understand the drivers of population dynamics.

The reproductive biology of flatfish has been described for numerous species (Rijnsdorp and Witthames 2005). However, there is a scarcity of information for flatfish species in the southern

hemisphere. The greenback flounder, *Rhombosolea tapirina*, occurs in estuaries and nearshore habitats along the southern coast of Australia and also in New Zealand (Gomon et al. 2008), where it supports local commercial and recreational fisheries (Kailola et al. 1993). In South Australia, *R. tapirina* has been regarded as an estuarine resident, i.e. it completes its entire life cycle within estuaries (Hall 1984), despite a superficial understanding of its population dynamics. Early studies identified numerous consistencies in the reproductive biology of *R. tapirina* such as iteroparity, multiple batch spawning and asynchronous oocyte development (Kurth 1957; Barnett and Pankhurst 1999). However, differences have also been found which include the timing of gonad development in relation to water temperature (Kurth 1957; Crawford 1984). Furthermore, information on size and age of sexual maturity, fecundity and spawning frequency for *R. tapirina* and how these parameters are influenced by the environment is not available.

This study investigated the reproductive biology of *R. tapirina* in the estuary of Australia's largest river system, the Murray-Darling, in South Australia. The Coorong estuary supports a commercial fishery which accounts for >95 % of the State's total annual catch of *R. tapirina* (Ferguson 2012). Since the 1980s, commercial catch from the estuary has been characterised by high inter-annual variation and ranged from 0.1 to 63.3 t.yr^{-1} (Ferguson 2012). Record low catches during the 2000s averaged 3.9 t.yr^{-1} and coincided with the most severe hydrological drought to affect south-eastern Australia since the 19th century (Leblanc et al. 2012). During the drought, the minimal freshwater inflow to the estuary resulted in hypersaline conditions throughout most of the system. It is unknown how this may have affected the reproductive biology of *R. tapirina*. Such knowledge may help explain the temporal variation in catch and the recent decline in population abundance (Ferguson 2012).

The primary aim of this study was to describe the reproductive characteristics and temporal variation in gonad development and spawning activity of *R. tapirina* and to relate these to water temperature and salinity. To achieve this, several parameters were determined for two areas characterised by different salinity regimes over a two-year period. The specific objectives were to: (1) determine the timing of gonad development and compare this to water temperature; (2) determine the reproductive mode of *R. tapirina* and to test the hypothesis that the species is a multiple batch spawner with asynchronous oocyte development; (3) determine the timing of spawning by analysing the microscopic characteristics of ovaries to differentiate spawning and non-spawning fish and compare this to water temperature and between two areas with contrasting

salinity regimes; (4) determine size and age at sexual maturity; and (6) estimate batch fecundity and to determine the relationship with fish size.

4.2   Materials and methods

4.2.1   Study region

The study was conducted in the Coorong estuary, South Australia (Figure 4.1). The Murray River is a heavily regulated system, whereby a series of tidal barrages limit the amount of freshwater discharged into the Coorong estuary (Figure 4.1). Prior to the installation of tidal barrages in the early 1940s, average annual freshwater discharge to the estuary was 12 233 GL (CSIRO 2008). Since then, less than 40% of the natural average discharge has reached the estuary in each year (CSIRO 2008).
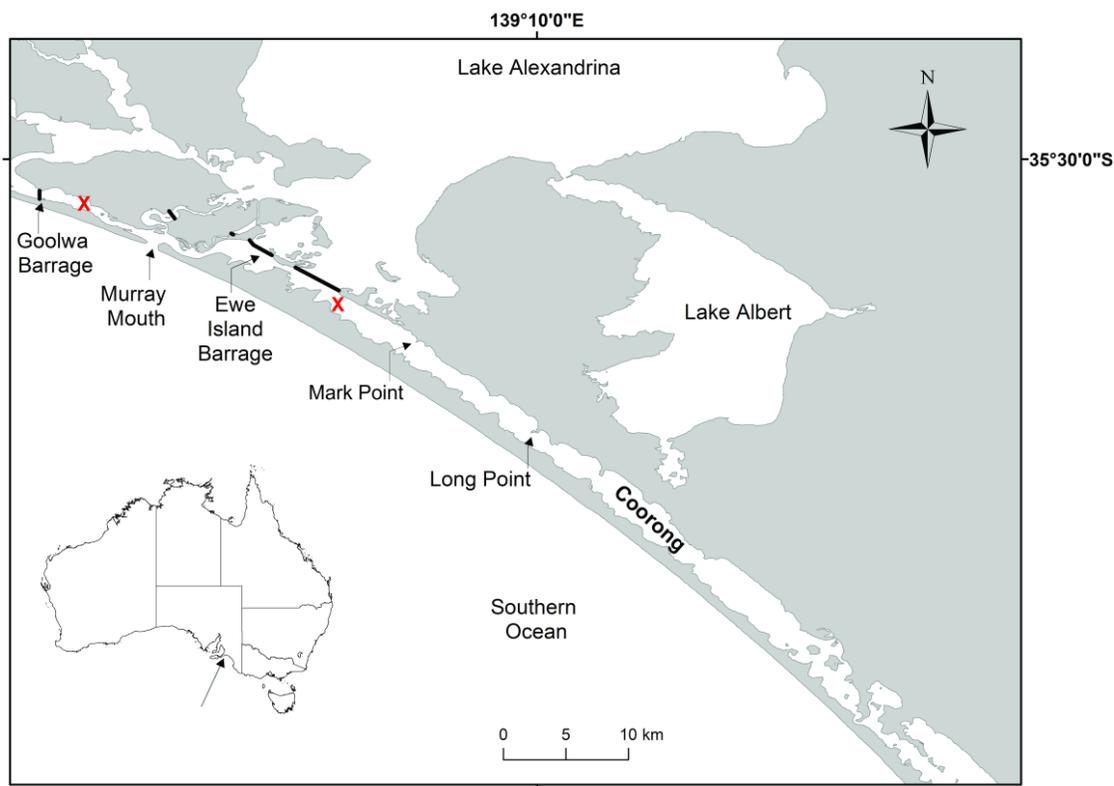
Figure 4.1 Map of the Coorong estuary, showing the two study areas: the Estuary, i.e. from Goolwa Barrage to Ewe Island Barrage and North Coorong, i.e. from Ewe Island Barrage to Mark Point. The location of the water monitoring beacon for each study area is also shown (X). Inset shows location of study region relative to Australia. The locations of the barrages that separate the estuarine system from Lake Alexandrina are indicated by black lines.

During the recent Millennium Drought from 2002 to 2010 (Leblanc et al. 2012), freshwater discharge into the Coorong estuary was minimal. This included a 3-year period from 2007 to August 2010, when no freshwater discharged into the estuary. The lack of flow during this period contributed to a southward longitudinal salinity gradient from the mouth of the estuary (Webster 2010). As a consequence, salinity increased and ranged from marine (30 – 36 g L $^{-1}$) near the Murray Mouth, to hypersaline (>120 g L $^{-1}$) in the southern parts of the Coorong (Kingsford et al. 2011). High salinity in the southern Coorong caused extensive alteration and loss of critical fish habitat, resulting in a significant spatial contraction in habitat for many fish populations including for *R. tapirina* (Ye et al. 2011; Overton and Doody 2013).

4.2.2   Environmental variables

Water temperature and electrical conductivity (EC) data were collected from two locations in the Coorong estuary from January 2009 to December 2010 (Figure 4.1). EC was used as a proxy for salinity and is referred to as salinity hereafter (freshwater = 0 µS/cm; seawater = 50 000 µS/cm). At each location, water temperature was recorded at 2-hourly intervals by a data logger (HOBO® Pendant Temperature Data Logger) attached to a navigation beacon one metre below the low tide mark. Salinity was recorded hourly by a continuous water monitoring station attached to each beacon by the Department of Environment, Water and Natural Resources (Government of SA 2011).

4.2.3   Sample collection and processing

In total, 735 *R. tapirina* were collected between January 2009 and December 2010 from two areas in the Coorong estuary. One area, the Estuary, included all waters downstream of the Goolwa Barrage to the Murray Mouth (Figure 4.1). The other, North Coorong, extended from the Ewe Island Barrage to Mark Point. Fish were collected from commercial fishers who targeted *R. tapirina* with 4.75″ mesh gill nets. Scientific sampling was also done using 3.5″ mesh haul nets and a hand-held dip net. Sampling effort and catch rates varied throughout the study period (Table 4.1). In the Estuary, fish were collected in most months in 2009 and 2010, whereas for the North Coorong, sampling effort was low in 2009 and fish were collected in most months in 2010 (Table 4.1). In each year, sampling effort increased as soon as spawning fish were encountered in order to describe the fine-scale temporal variation of spawning.

Most fish were processed within 8 hours of capture. Each fish was measured for total length (TL) to the nearest millimetre and weighed to the nearest 0.01 g. The sagittae, i.e. the largest pair of

otoliths, were removed, cleaned, dried and stored. Later, the left otolith was prepared for ageing (Chapter 3). The gonads were removed, examined to determine the sex of the fish, and weighed to the nearest 0.01 g. Gonadosomatic indices (GSI) were calculated to assess the timing and duration of the gonad development, as: $GSI = [W_g/W_f]*100$, where W_g is gonad weight and W_f is gonad-free fish weight. The relationship between water temperature and gonad development for female *R. tapirina* was measured using Pearson's correlation analysis ($\alpha = 0.05$) between estimates of GSI and mean monthly water temperature. Correlation analysis was not done for males due to the small sample sizes.

Table 4.1 Summary of the samples of *R. tapirina* collected in 2009 and 2010 from the Estuary and North Coorong for analysis of reproductive characteristics. Sample sizes do not include the number of immature fish (*n*=72), as these were only used to estimate size and age at maturity. Sampling method: G – gill net; H – haul net; D – dip net.

Month-Year	Estuary			North Coorong		
	No. of samples	Sample size (*n*)	Sampling method	No. of samples	Sample size (*n*)	Sampling method
Jan-09	1	6	D	1	1	D
Feb-09	-	-	-	1	18	H
Mar-09	1	8	D	-	-	-
May-09	3	14	G	-	-	-
Jun-09	2	24	G	-	-	-
Jul-09	3	13	G	1	26	H
Aug-09	3	29	G	-	-	-
Oct-09	2	12	G	-	-	-
Nov-09	1	19	G	-	-	-
Jan-10	1	5	D	1	2	D
Feb-10	1	24	H	1	15	H
Mar-10	2	33	H	3	43	GH
Apr-10	1	8	D	2	24	GH
May-10	5	60	GH	1	26	H
Jun-10	1	26	H	1	21	H
Jul-10	1	23	H	1	16	H
Aug-10	2	31	GH	1	15	H
Sep-10	6	49	GH	1	1	G
Oct-10	2	38	GH	1	3	H
Nov-10	1	1	D	1	19	H
Dec-10	1	10	H	-	-	-
Total	40	433		17	230	

To determine the timing and duration of gonad development, ovaries were classified macroscopically to one of five stages of development based on the size, colour and visibility of oocytes (Table 4.2). For males, testes were examined macroscopically and classified into those that showed signs of development and those that did not (Table 4.2).

All ovaries that were classified to Stages 3, 4 and 5 and some to Stages 1 and 2 ($n = 407$), were subjected to more detailed analysis by histological preparation and microscopic examination to assess reproductive mode and spawning activity for *R. tapirina*. For these ovaries, a cross-section was removed from the centre of one ovary, placed into an embedding cassette and preserved in 10% formalin. This was also done for all testes ($n = 26$). For ovaries that were classified as Stage 4, the second ovary was used to produce size frequency distributions of oocytes and to estimate batch fecundity. These ovaries were split open longitudinally and the oocytes were washed out from the ovary, collected in a 100 µm sieve, and transferred to a jar containing a fixative solution of 40 % formaldehyde, acetic acid and calcium chloride (FAACC) for preservation (Lowerre-Barbieri and Barbieri 1993).

Table 4.2 Macroscopic characteristics of ovaries and testes of *Rhombosolea tapirina* at progressive stages of development, based on Barnett and Pankhurst (1999).

Sex	Macroscopic stage	Macroscopic characteristics
Female	1 (Immature)	Sex often indistinguishable; ovaries small, undeveloped, narrow, firm, translucent/blackish/grey threads often with grey sheen.
	2 (Developing/resting)	Ovaries small/medium; opaque pink/red/grey in colour; individual oocytes difficult to discern, often not visible.
	3 (Developed/ripe)	Ovaries large and plump; yellow/cream in colour; opaque oocytes easily discernible but not translucent.
	4 (Gravid/ovulating)	Ovaries large and turgid; yellow/cream in colour; hydrated/translucent oocytes visible among opaque oocytes through epithelium; oocytes may be ovulated; blood vessels larger and more defined.
	5 (Spent/regressing)	Ovaries medium/small; deep red/orange in colour; generally limp/flaccid with shrunken appearance; some residual large oocytes may remain; ovary often bloodshot with regressed blood vessels.
Male	1 (Immature)	Sex often indistinguishable; testes small, undeveloped, narrow, firm, blackish/grey threads often with grey sheen.
	2 (Developed)	Testes small/medium and shorter in length relative to ovaries; pink/greyish in colour with grey/gold sheen.

4.2.4   Laboratory analysis of preserved samples

Histological sections were prepared from the formalin-preserved gonad tissue for microscopic analysis. Each tissue sample was removed from the formalin solution and immersed in a series of ethanol baths of progressively increasing concentrations of alcohol to dehydrate the tissue. Tissue was then embedded in a hard paraffin block for sectioning. It was sectioned at 7-8 μm, stained with haematoxylin and eosin and mounted on microscope slide. Each section was examined using a compound microscope at 100x magnification and classified according to the most advanced stage of oocyte development, level of atresia, and presence/absence of post-ovulatory follicles (POFs). The oocytes were classified as either unyolked, partially yolked, advanced yolked, migrating nucleus or hydrated (Table 4.3). The frequency of atretic oocytes in relation to the number advanced yolked oocytes was also recorded, along with the presence/absence of POFs.

Table 4.3 Descriptions of the different stages of development of oocytes, atretic oocytes and post-ovulatory oocytes. Images of oocytes, POFs and atretic oocytes are shown in Figures 4.6 and 4.7.

Microscopic stage	Macroscopic characteristics
1. Unyolked	Small undeveloped oogonia embedded in ovigerous tissue; large nucleus surrounded by a thin layer of cytoplasm; cytoplasm densely stained basophilic with no vacuoles; several nucleoli along nucleus periphery.
2. Partially yolked	Similar to Stage 1, but slightly larger; thicker layer of cytoplasm surrounding the nucleus; nucleus and cytoplasm become increasingly eosinophilic; cortical alveoli (lipid droplets) form several peripheral rows in cytoplasm; follicle layer comprises two cell layers; zona radiata present but thin.
3. Advanced yolked	Larger than Stage 2. Cortical alveoli fuse together becoming larger and coalesce toward centre; eosinophilic yolk globules link to form granular mass and completely fill cytoplasm; nucleus still central; zona radiata thick and highly eosinophilic.
4. Migratory nucleus	Similar to Stage 3, except nucleus has migrated, or is migrating toward the peripheral cytoplasm; nucleus loses its integrity and begins to breakdown; cortical alveoli and yolk globules continue to coalesce. This stage represents the initiation of the hydration process.
5. Hydrated	Rapid increase in size due to uptake of fluid (hydration); irregular shape due to stretching and thinning of zona radiata; breakdown of nucleus is complete; yolk plates and cortical alveoli have fused to form homogenous mass. Spawning is imminent – leading to the release of the oocyte into the ovarian lumen.
Post-ovulatory follicle (POF)	Follicular cell layer has ruptured and collapsed after the release of the oocyte; highly convoluted with a distinct irregular lumen; features may occur at various stages of degeneration.
Atretic oocyte	Zona radiata dissolves and fragments; nucleus becomes irregular in shape and more basophilic; yolk granules lose their integrity and dissolve.

Based on the microscopic examination of the sectioned ovarian tissue, each female was classified as either spawning or non-spawning. Ovaries from spawning fish contained oocytes in the migratory nucleus stage, and/or the hydrated stage, and often also contained POFs. Non-spawning fish had ovaries that contained partially yolked oocytes and/or advanced yolked oocytes, but no oocytes at the migratory nucleus stage, hydrated stage, or POFs.

4.2.5   Reproductive mode

To determine the reproductive mode of *R. tapirina*, frequency distributions of oocyte sizes were calculated from sections of ovaries from several fish - one that had been classified to Stage 2 and one to Stage 3 according to Table 4.2. Sections were viewed under a compound microscope using image analysis software (Optimas^{TM} v5.1), which displayed the image of the ovarian tissue on a computer screen via a camera mounted on the microscope. A transect was randomly imposed on a still image of the matrix of the oocytes. Oocytes that contained a clearly visible nucleus were measured to determine their diameter and classified as either unyolked, partially yolked or advanced yolked. This process was repeated until at least 150 oocytes had been measured for each of the two fish. A frequency distribution of oocyte size was generated for both fish, differentiating the different developmental stages. This method could not be used to measure the diameter of hydrated oocytes, as the integrity and shape of these oocytes often became distorted by the sectioning process. As such, a frequency distribution of whole oocytes was also produced for one fish with Stage 4 ovaries. For this, ten 1 ml sub-samples from the batch of preserved oocytes, i.e. those preserved for estimating fecundity, were pipetted out and placed in an egg counting tray. Diameters of whole oocytes were then measured using a dissecting microscope at 20x magnification and a calibrated ocular micrometer.

4.2.6   Spawning activity

To examine temporal and spatial variation in spawning activity, the number of mature females classified as either spawning or non-spawning, based on the microscopic analysis of oocytes in their ovaries was determined. Estimates of spawning fraction (S_{fr}) reflected the proportion of mature females that were spawning at the time of capture and were estimated using the equation: $S_{fr} = N_s/N_m$, where N_s = the total number of spawning females, and N_m = the total number of mature females in the sample. Spawning interval (S_i) was also determined to estimate the average number of days between spawning events using the equation: $S_i = 1/ S_{fr}$ (DeMartini and Fountain 1981). Spawning fraction and interval were calculated for samples that contained $\geq$ 3 fish (Table 4.1), and for those collected in 2010 only, as ovaries collected in 2009 were not preserved for

histology. The relationship between water temperature and spawning activity for female *R. tapirina* was considered by correlation analysis (Pearson, $\alpha = 0.05$) between monthly estimates of spawning fraction and mean monthly water temperature.

To investigate the potential influence of salinity on spawning activity, monthly estimates of spawning fraction were calculated and compared between the two study areas with different salinity regimes. A chi-square contingency test was done on the numbers of spawning and non-spawning females sampled in each area in each month from March to October 2010. All statistical analyses were run using SPSS v.20 for Windows (SPSS Inc., Chicago,Illinois, USA).

4.2.7 Maturity

To determine the length (SOM_{50}) and age (AOM_{50}) at which 50 % of the sampled population were sexually mature, fish samples from the two study areas were pooled. Fish with gonad development stages of Stage 2 and higher were deemed to be sexually mature (Table 4.2). Logistic curves were fitted to describe the proportion of sexually mature individuals (P_m) by TL using the equation: $P_m = 1/(1 + \exp[-r(L - L_m)])$, where r is the rate of fish maturity (slope), L is the TL of fish, and L_m is the mean length at which 50 % of the sampled population is sexually mature. Logistic curves were also fitted to describe the proportion of sexually mature individuals by age (months) using: $P_m = 1/(1 + \exp[-r(A - A_m)])$, where r is the rate of fish maturity (slope), A is fish age, and A_m is the mean age at which 50 % of the sampled population is sexually mature. The Solver routine in MS Excel™ was used to estimate optimal L_m and r values.

4.2.8 Fecundity

Batch fecundity was assessed based on the number of hydrated oocytes present in ovaries classified to Stage 4 (Table 4.2). Each batch of oocytes was removed from the FAACC solution, rinsed with water using a 100 μm filter, and placed in water, making up the volume to 1 L. This 1 L sample was agitated to distribute the oocytes evenly. Then ten 1 mL sub-samples were pipetted out, placed in an egg counting tray, and examined under a binocular microscope using transmitted light. For each sub-sample, hydrated oocytes (>600 μm) were counted. The average number of hydrated oocytes per ml was calculated. Total batch fecundity was estimated by multiplying the average number of oocytes per ml to the full sample of 1000 ml and multiplying by two, to account for both ovaries.

4.3 Results

4.3.1 Environmental Parameters

There was distinct seasonality in water temperature in the two study areas. In the Estuary, water temperature was highest in December and January of each year, reaching annual maxima of 27.8 ^{o}C and 27.3 ^{o}C in 2009 and 2010, respectively. From January to June in each year, temperature gradually declined to below 11°C and remained low until early August (Figure 4.2A). In North Coorong, water temperature conformed to the same seasonal pattern as for the Estuary, although was more variable (Figure 4.2B).
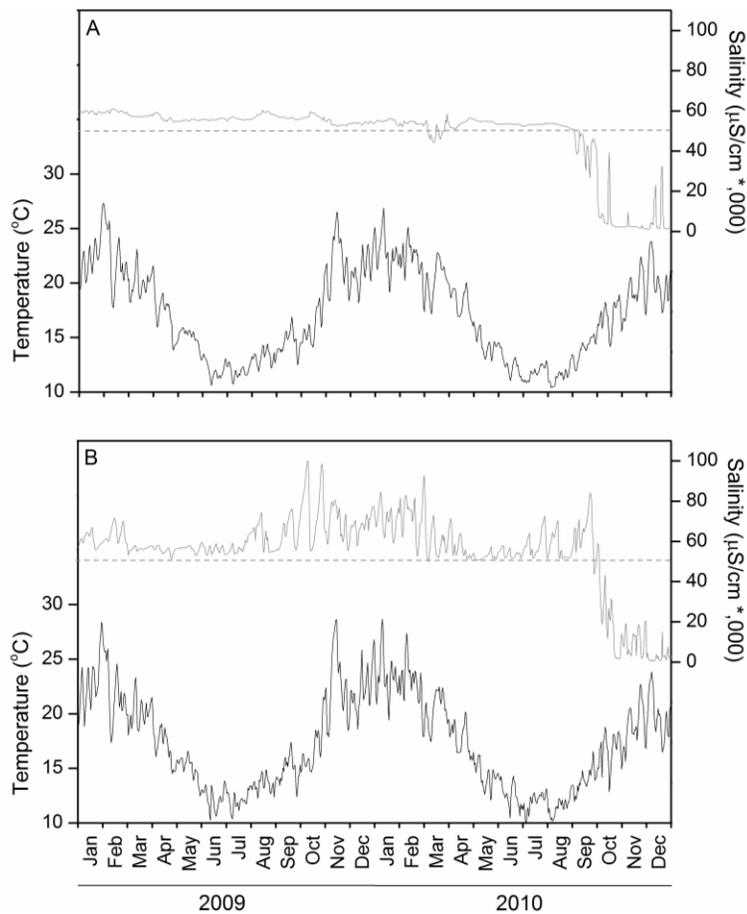
Figure 4.2 Daily mean water temperature (black line) and salinity (grey line) for (A) the Estuary and (B) North Coorong, from January 2009 to December 2010. Salinity data were obtained from the Department of Environment, Water and Natural Resources (Government of South Australia 2013). Broken grey line shows the approximate salinity of seawater, i.e. 50 000 μS/cm.day^{-1}.

In the Estuary, salinity was relatively stable from January 2009 to mid-2010 and averaged 55 251 µS/cm.day^{-1} (range: 44 248 and 61 087µS/cm.day^{-1}) (Figure 4.2A). In late September 2010, drought-breaking freshwater inflows from the Murray River recommenced and reduced salinity to < 8000 µS/cm.day^{-1}, where it remained on most days until late December. In the North Coorong, salinity was highly variable from January 2009 to the commencement of freshwater inflows in September 2010 and typically higher than the Estuary. During that period, salinity ranged from 49 973 µS/cm.day^{-1} to 99 960 µS/cm.day^{-1}, and averaged 61 513 µS/cm.day^{-1}. The high variation was the likely consequence of regular wind-driven surges of hypersaline water moving along the longitudinal gradient of the estuary from the south east. In September 2010, salinity decreased and remained < 20 000 µS/cm.day^{-1} through to December.

4.3.2   Sample characteristics

For the Estuary, a total of 470 fish, ranging in size from 173 to 355 mm TL were considered for reproductive analyses (Figure 4.3A). A total of 87 % were females, 5 % were males, and the remaining fish were immature. For North Coorong, a total of 265 fish, ranging in size from 168 mm TL to 387 mm TL was collected (Figure 4.3B). A total of 80 % were females, 6% were males, and the remainder were immature. One hermaphrodite was also collected. For both areas, males and females were distributed throughout the size frequency distribution and the bias toward females in each area was generally consistent among months (Table 4.4).
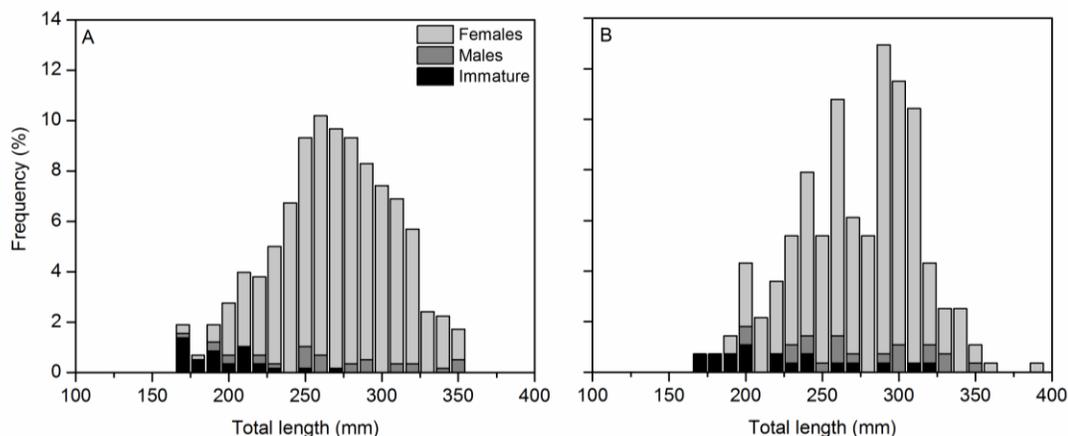
Figure 4.3 Length frequency by sex of *R. tapirina* considered for the analysis of reproductive biology from (a) the Estuary (n=470); and (b) North Coorong (n=265).

Table 4.4 Summary of the characteristics of *R. tapirina* samples collected for the analysis of their reproductive biology between January 2009 and December 2010 in the Estuary and North Coorong. In March 2010, one hermaphrodite was collected in North Coorong.

| Month | Estuary | | | | North Coorong | | | |
| | 2009 | | 2010 | | 2009 | | 2010 | |
	Females	Males	Females	Males	Females	Males	Females	Males
Jan	5	1	5	-	-	1	1	1
Feb	-	-	23	1	18	-	14	1
Mar	8	-	32	1	-	-	40	2
Apr	-	-	7	1	-	-	23	1
May	13	1	59	1	-	-	23	3
Jun	24	-	25	1	-	-	20	1
Jul	12	1	22	1	25	1	14	2
Aug	-	1	30	1	-	-	13	2
Sep	28	-	43	6	-	-	-	1
Oct	11	1	35	3	-	-	3	-
Nov	19	-	-	1	-	-	18	1
Dec	-	-	8	2	-	-	-	-
Total	120	5	289	19	43	2	169	15

4.3.3 *Timing and duration of gonad development*

<u>Gonadosomatic indices</u>

GSI estimates were used to describe the timing and duration of gonad development for *R. tapirina.* In 2009, GSIs for females from the Estuary increased from an annual low in January, were high from May to July, and gradually decreased from August to November (Figure 4.4A). In 2010, GSIs for females were lowest in January and February and increased sharply to the annual maximum in June. It remained high in July, before decreasing in following months until December. A similar temporal pattern was evident for males.

For North Coorong in 2009, a lack of samples limited the analysis of temporal variation in GSIs, although the estimated values for both sexes were relatively high in July (Figure 4.4B). In 2010, GSIs for both sexes were lowest in January and February. From then, female GSIs increased and were highest from April to June and decreased in the following months to November. Similarly, GSIs for males increased in March, peaked in April, remained relatively high until June and subsequently declined in each month to November.
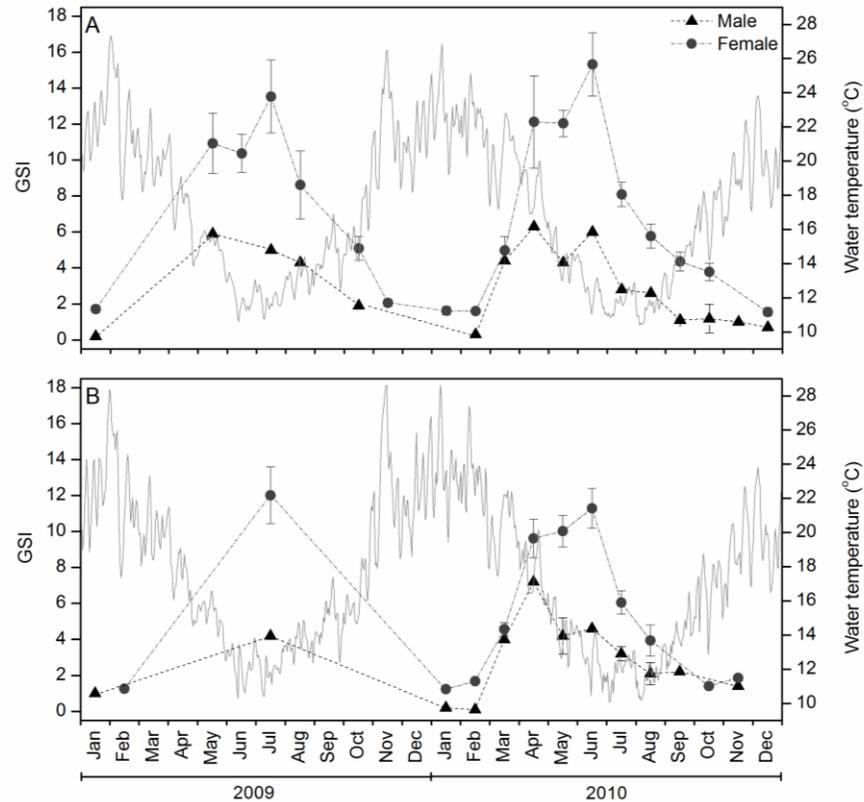
Figure 4.4 Mean gonadosomatic indices (±SE) for males and females by month for (A) the Estuary and (B) North Coorong with logged daily maximum water temperature (solid grey line) overlaid.

There was a strong negative relationship between water temperature and female GSI for the Estuary (r = -0.715, P < 0.001) and North Coorong (r = -0.666, P = 0.018). In each area, the development of ovaries commenced in March 2010 when the daily maximum water temperature had declined to approximately 20.5 °C. GSIs for both sexes, increased in subsequent months until June as water temperature decreased to 12 °C. As water temperature increased from July, estimates of GSI decreased indicating the slowing of gonad development. GSIs continued to decrease to November/December, as water temperature increased to > 20 °C.

Macroscopic staging of ovaries
Macroscopic staging of ovaries also indicated a seasonal pattern of gonad development in each year (Figure 4.5a). For the Estuary, the proportion of mature resting females (Stage 2) was highest during the summer months of both years. In March and May 2009, >70 % of ovaries were developed/ripe (Stage 3). Fish at Stage 4 were present in July, August and October. The first regressing (Stage 5) ovaries were recorded in June and were evident in most months to November. By November, 83 % of fish were resting. In 2010, the development of ovaries followed a similar temporal trend, i.e. Stage 3 fish were common from March to June, Stage 4

fish were recorded from May to September and the incidence of Stage 5 fish was highest in September and October. Macroscopic staging indicated that gonad development of females in the Estuary in each year occurred from March through to November, and spawning occurred from May to October.
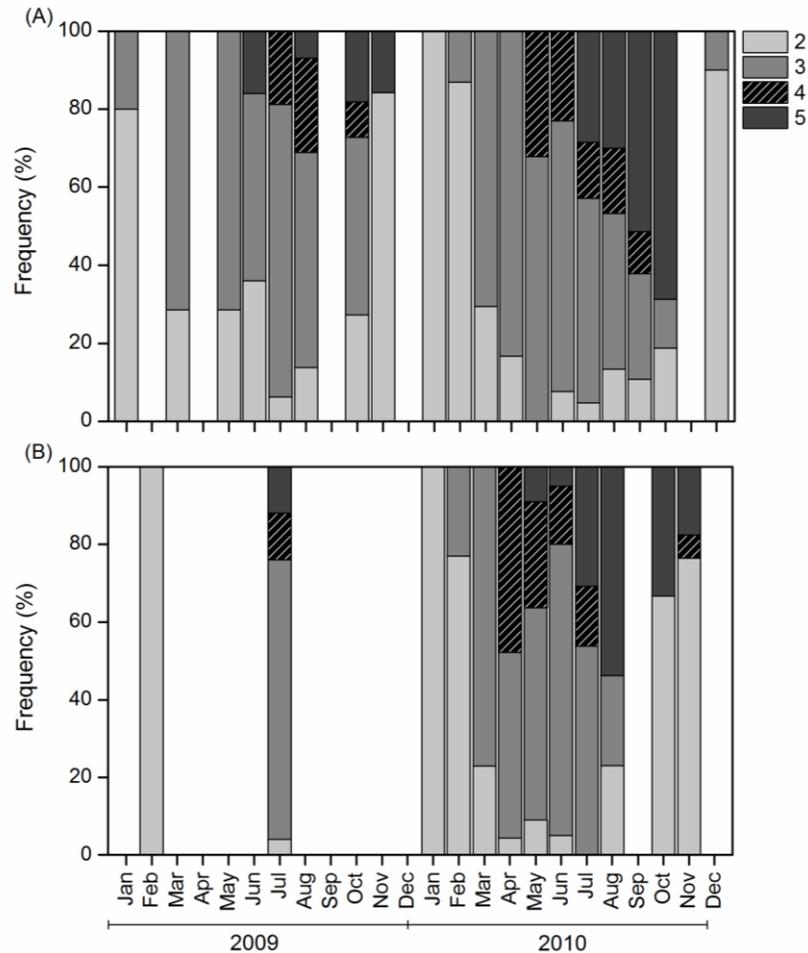
Figure 4.5 Percentages of female macroscopic stages by month for (A) the Estuary and (B) North Coorong. See Table 4.2 for macroscopic staging criteria.

For the North Coorong in 2010, all mature females in January were resting (Figure 4.5b). The frequency of Stage 3 fish increased in February and March, and by April 48 % of females were Stage 4. Stage 4 fish were evident in each month thereafter to July. In August, 54 % of ovaries were spent. Overall, the period of gonad development for females in the North Coorong, commenced in April and continued until November, with spawning occurring in most months from April to November.

4.3.4 Description of microscopic characteristics of ovaries

Analysis of histological preparations of ovaries indicated that oocytes at a range of developmental stages and post-ovulatory follicles co-occurred in ovaries of mature fish (Figure 4.6). While ovaries of immature fish were represented by a tight matrix of exclusively unyolked oocytes (Figure 4.6A), ovaries of Stage 2 fish contained a tight arrangement of unyolked and partially yolked oocytes. No atresia was present in either Stage 1 or Stage 2 ovaries.
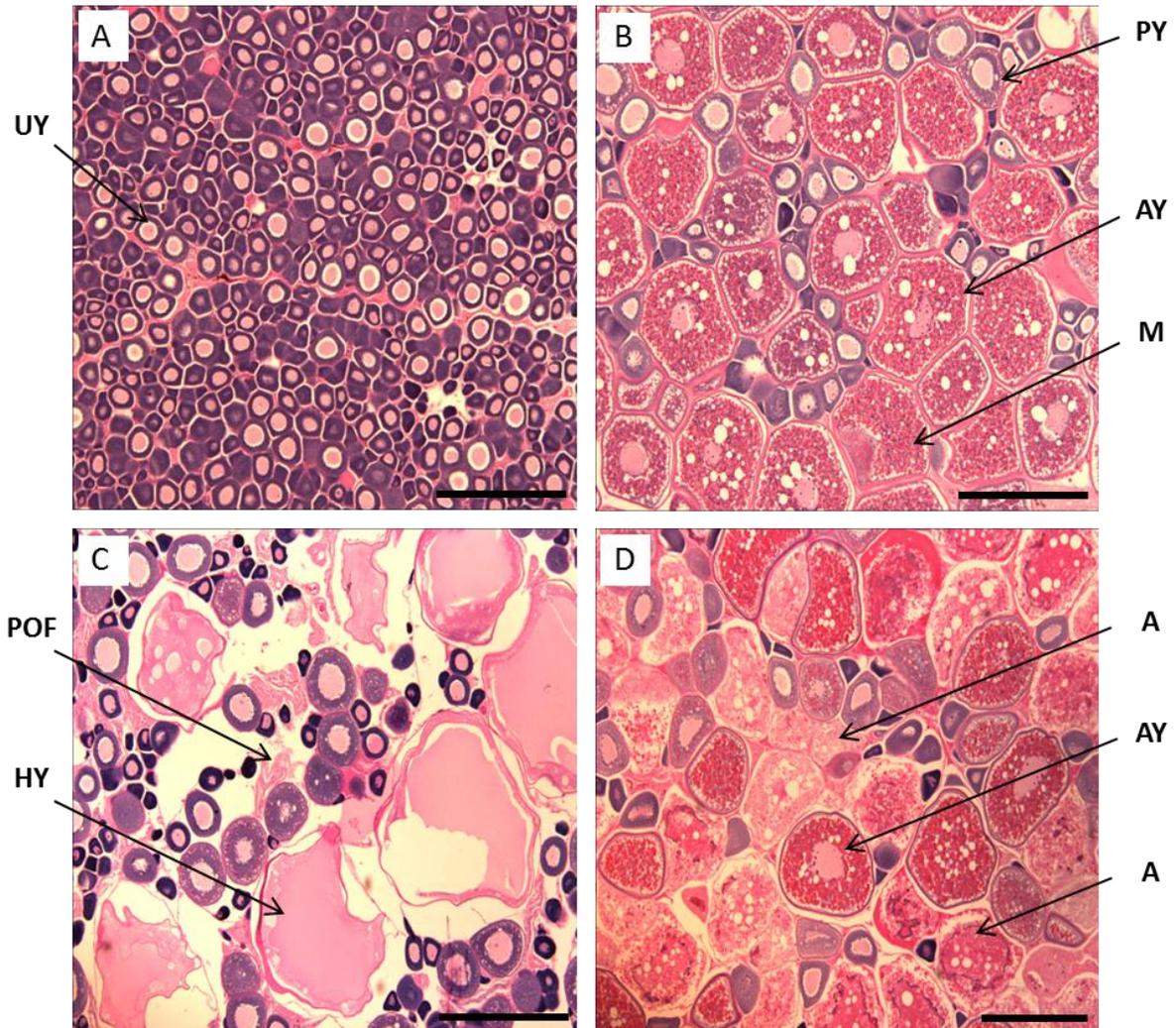
Figure 4.6 Histological sections of ovaries, showing oocytes at different stages of development: (A) ovary of an immature fish comprising a tight matrix of unyolked oocytes; (B) ovary with unyolked, partially yolked and numerous advanced yolked oocytes; (C) ovary with several hydrated oocytes and POFs; (D) ovary with numerous atretic oocytes and advanced oocytes (UY – unyolked; PY – partially yolked; AY – advanced yolked; M – migrating nucleus; POF – post-ovulatory follicle; HY – hydrated; A – atretic; black scale bars - 500 μm).

Stage 3 ovaries contained unyolked, partially yolked and advanced yolked oocytes generally arranged in a tight matrix (Figure 4.6B), although some were loosely packed. Stage 4 ovaries were characterised by the presence of hydrated oocytes, but also displayed unyolked, partially yolked and advanced yolked oocytes (Figure 4.6C). Some Stage 4 ovaries also had oocytes with a migrating nucleus or POFs. The oocyte matrix of Stage 4 ovaries was generally tightly packed due to the presence of hydrated oocytes, but was often more open than the earlier stages. Some minor atresia was often evident.

Stage 5 (spent/regressing) ovaries often contained unyolked, partially yolked and advanced yolked oocytes, but were dominated by a high incidence of (>50 %) of atresia (Figure 4.6D). The density of the oocyte matrix was highly variable. Hydrated oocytes and POFs were not present in Stage 5 ovaries.

4.3.5   *Reproductive mode*

The co-occurrence of oocytes at a range of developmental stages and sizes in the ovaries of mature fish was examined to determine the reproductive mode of *R. tapirina.* Unyolked oocytes ranged in size from 36 to 138 μm and overlapped in diameter with partially yolked oocytes, which ranged from 89 to 210 μm (Figure 4.7A). Advanced yolked oocytes ranged from 209 to 418 μm, overlapping in size with larger partially yolked oocytes (Figure 4.7B). The size distributions were continuous across developmental stages.

The size distribution of preserved whole oocytes from Stage 4 fish was bimodal, with one mode relating to non-hydrated oocytes and the other to larger hydrated oocytes (Figure 4.7C). Since all oocytes were opaque it was not possible to determine whether the non-hydrated oocytes were unyolked, partially yolked or advanced yolked oocytes. Non-hydrated whole oocytes ranged from 40 to 504 μm. The size distribution of non-hydrated oocytes was continuous and contained no clear gaps, whilst there was a clear gap to hydrated oocytes, which were much larger and ranged in size from 638 to 889 μm.
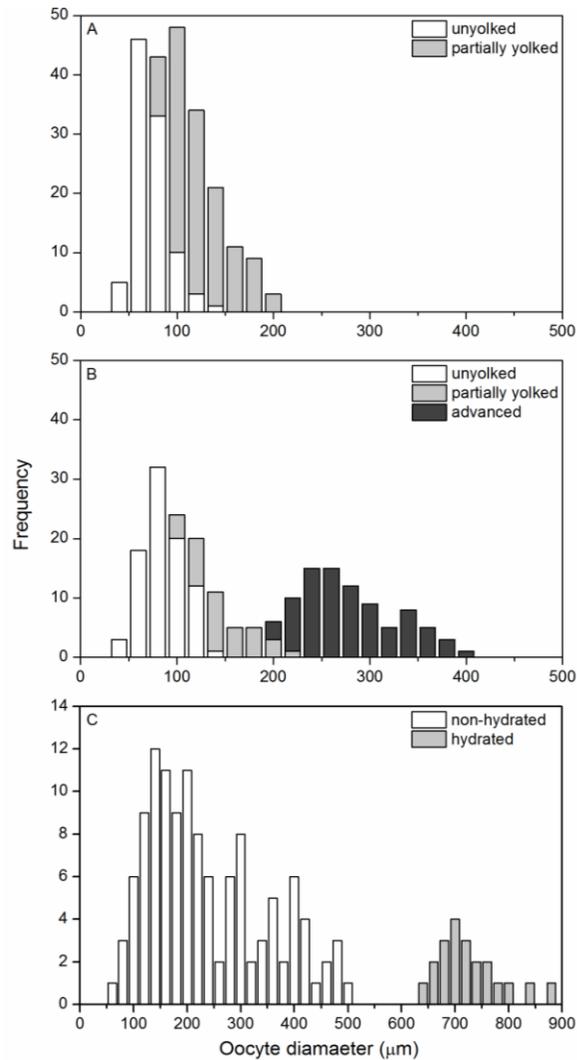
Figure 4.7 Size-frequency distributions of oocytes at different stages of development, measured from (A) a sectioned ovary of a Stage 2 fish ($n = 220$ oocytes); (B) a sectioned ovary of a Stage 3 fish ($n = 207$) and (C) whole preserved oocytes from the ovaries of a Stage 4 fish ($n = 142$).

4.3.6   Spawning activity

For the Estuary, spawning females were first collected in late March (Table 4.5). The estimate of spawning fraction for this sample was 0.06 and then increased for samples collected in April and early May when it ranged from 0.21 to 0.38 (Table 4.5). Spawning fraction increased and peaked at 0.71 in mid-May, remained relatively high to mid-September, and decreased to 0.12 in October. No spawning fish were sampled from mid-October onwards. Therefore, microscopic analyses confirmed that spawning in the Estuary occurred over an 8-month period from March to October. For the North Coorong, spawning fraction followed a similar temporal cycle, i.e. spawning was first detected in March, was highest in May, and continued until at least late August (Table 4.5). None of the three females sampled in October were spawning.

Table 4.5 Estimates of spawning fraction and spawning interval in days for samples collected in the Estuary and North Coorong that contained spawning females in 2010.

Study area	Sample date	No. of mature females	No. of spawning females	Spawning fraction	Spawning interval (days)
Estuary	24 Mar	32	2	0.06	16.7
	30 April	7	2	0.29	3.5
	2 May	8	3	0.38	2.7
	4 May	11	3	0.27	3.7
	10 May	9	6	0.67	1.5
	12 May	7	5	0.71	1.4
	20 May	24	5	0.21	4.8
	22 June	25	6	0.24	4.2
	28 July	22	7	0.32	3.1
	23 Aug	14	7	0.5	2
	24 Aug	16	10	0.63	1.6
	15 Sept	10	6	0.6	1.7
	17 Sept	10	5	0.5	2
	18 Sept	13	2	0.15	6.5
	24 Sept	10	2	0.2	5
	1 Oct	33	4	0.12	8.3
	14 Oct	2	0	-	-
North Coorong	24 Mar	40	2	0.05	20
	15 April	13	4	0.31	3.3
	22 April	10	4	0.4	2.5
	20 May	23	11	0.47	2.1
	22 June	20	5	0.25	4
	28 July	14	4	0.29	3.5
	23 Aug	13	2	0.15	6.5
	14 Oct	3	0	-	-
	15 Nov	18	0	-	-

Estimates of spawning fraction were used to estimate spawning interval, i.e. the number of days between spawning events at the different times through the spawning season. In late March, females in the Estuary spawned, on average, once every 17 days (Table 4.5). By late April, fish spawned, on average, every 4 days. This relatively high frequency of spawning was maintained until mid-September, with spawning occurring every two days in early May, late August and mid-September (Table 4.5). By early October, spawning interval had increased again to, on average, every 8 days, after which no subsequent spawning was detected. A similar temporal cycle was evident for females from North Coorong, although spawning occurred less frequently in most months and was not detected from late August onwards (Table 4.5).

For the Estuary, there was a strong negative correlation between average monthly water temperature and spawning fraction (r = -0.865, P < 0.001). Spawning females were first detected in March when the average temperature was 20.2 °C, and their frequency increased as temperatures declined (Figure 4.8A). Spawning fraction was highest in August when the water temperature averaged 11.5 °C. As water temperature increased in September and October, the proportion of females that were spawning declined. No spawning activity was detected in November and December when water temperature averaged 19 °C and 20.3 °C, respectively.
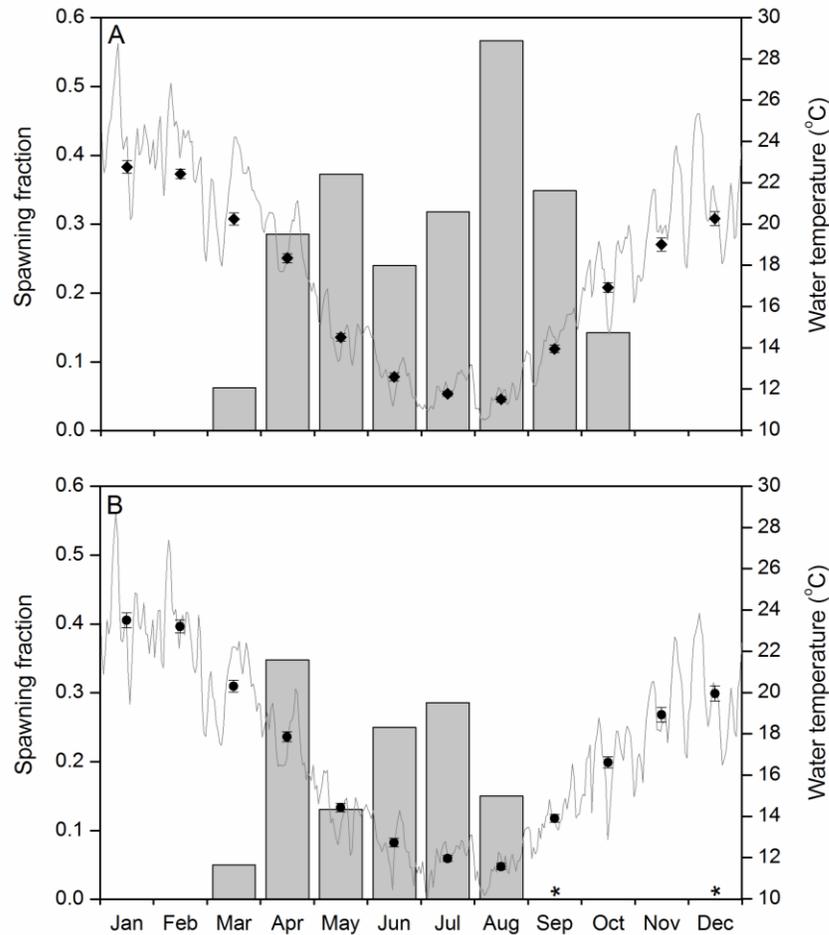
Figure 4.8 Monthly spawning fractions (bars) for the (A) Estuary and (B) North Coorong in 2010 with average monthly water temperature (black dots ± S.E.) and daily maximum water temperature (faint grey line) overlaid. * indicates months for which no samples were available.

For North Coorong, spawning females were first detected in March when the temperature averaged 20.3 °C (Figure 4.8B). Spawning fraction was highest in April when temperature averaged 17.9 °C and remained relatively high in each month to August, when the average water temperature was 12.7 °C. No mature females were sampled in September. By October, when the

average temperature had increased to 16.6 $^{\circ}$C, spawning was not evident amongst the three females sampled. The negative correlation between water temperature and spawning fraction was only marginally non-significant (r = -0.549, P < 0.064).

4.3.7   Spatial variation in spawning activity

To examine the potential influence of salinity on spawning activity, monthly estimates of spawning fraction were compared between the two study areas. Chi square contingency tests found no significant differences between the relative proportions of spawning and non-spawning fish between areas for each month of the spawning season (Table 4.6). One exception occurred in August, when the frequency of spawning females from the Estuary (57 %) was significantly higher than for North Coorong (15 %).

Table 4.6 Results of the 2 x 2 chi square contingency tests of the frequency of spawning and non-spawning females between the Estuary and North Coorong in 2010 (*significant at $P \leq 0.05$; ^{ns}: not significant at $P \geq 0.05$).

Month	Spawning status	Areas		Total	χ^2	*p*-value
		Estuary	**North Coorong**			
March	non-spawners	30	38	72	0.053	0.818^{ns}
	spawners	2	2			
April	non-spawners	5	15	30	0.019	0.891^{ns}
	spawners	2	8			
May	non-spawners	37	12	82	0.389	0.533^{ns}
	spawners	22	11			
June	non-spawners	19	15	44	0.053	0.817^{ns}
	spawners	6	4			
July	non-spawners	15	10	36	0.043	0.837^{ns}
	spawners	7	4			
August	non-spawners	13	11	43	6.267	0.012^{*}
	spawners	17	2			
September	non-spawners	28	-	43	No test	
	spawners	15	-			
October	non-spawners	31	3	38	0.383	0.536^{ns}
	spawners	4	0			

4.3.8   *Maturity*

The smallest mature female was 181 mm TL, while the smallest male was 190 mm TL (Figure 4.9). For females and males, SOM_{50} was 198 mm TL and 211mm TL, respectively. The youngest mature female was nine months of age, while the youngest male was 10 months of age (Figure 4.9). The AOM_{50} for females and males was 13 months and 14 months, respectively.
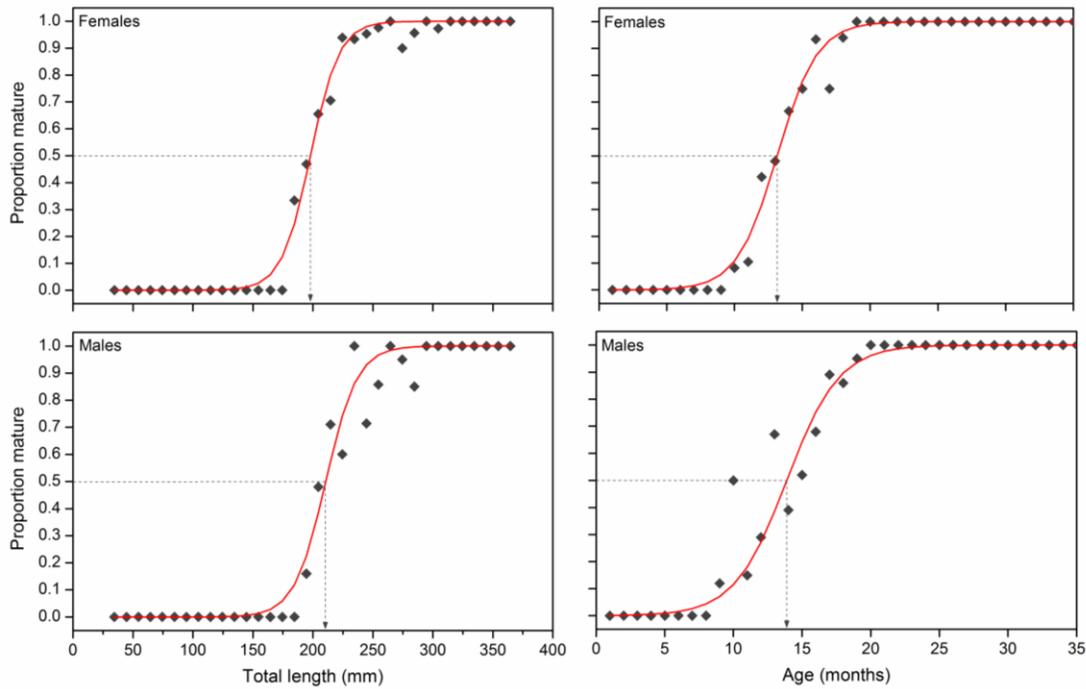
Figure 4.9 Proportion of sexually mature *R. tapirina* against the corresponding length (left) and age (right) intervals for females (n = 693) and males (n = 113) from the Estuary and North Coorong (pooled). Data points represent observed values.

4.3.9   *Fecundity*

Batch fecundity was estimated from the number of hydrated oocytes in the ovaries of fish classified to Stage 4. The estimates ranged from 3,600 to 113,200 oocytes (Figure 4.10). The relationships between batch fecundity and TL were linear for the Estuary and North Coorong. They were compared between areas by analysis of covariance, with no significant difference between slopes or intercepts (Table 4.7). As such, a single linear equation was produced to describe the relationship between batch fecundity and TL (mm): Fecundity = 539.6(TL) − 98115; r^2 = 0.68, P < 0.001, n = 55) (Figure 4.10).

Table 4.7 Results from the analyses of covariance comparing the linear relationships between batch fecundity (B_f) and fish size (TL) between the Estuary and North Coorong (*significant at $P \leq 0.01$; ns: not significant at $P \geq 0.05$).

Factor	n	df	F-ratio	P
slopes	35	1,51	1.061	0.31^{ns}
intercepts	20	1,52	2.940	0.09^{ns}
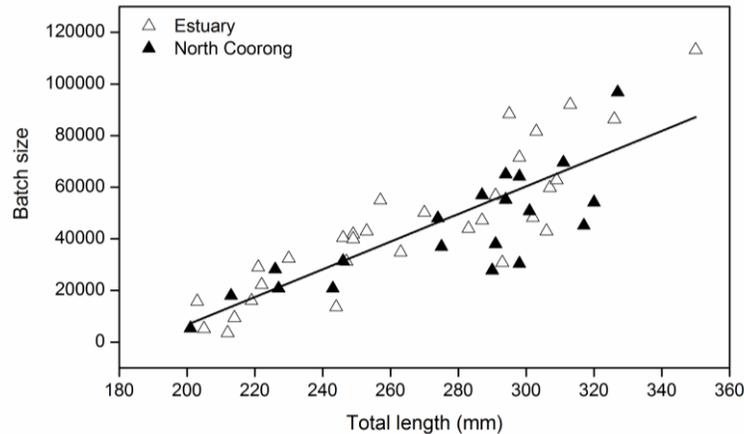
Figure 4.10 Plot of batch size vs. TL for the Estuary ($n = 35$) and North Coorong ($n = 20$) with regression line fitted.

4.4   Discussion

4.4.1   Timing of gonad development

Estimates of GSI and macroscopic staging indicated that gonad development for *R. tapirina* occurred across a protracted period from late March to October, with a peak between April and July. The initial rise in GSI for females occurred in March and related to a high incidence of fish with developed/ripe gonads. GSI was highest from April to July and coincided with a high frequency of fish with developed and gravid ovaries. The subsequent decline in GSI from August onwards was due to an increase in the frequency of fish with regressing and resting ovaries. Estimates of GSI for males followed a similar seasonal trend. This timing of gonad development for *R. tapirina* is similar to that of several populations in Tasmania, though development commenced one month earlier, i.e. in February, and finished one month later in November (Kurth 1957; Crawford 1984).

For most temperate fish species, water temperature has a strong influence on the timing of reproductive development (Pankhurst and Porter 2003). The results of this study are consistent

with this paradigm, as they demonstrate that the temporal variation in gonad development for *R. tapirina* was closely aligned with seasonal variation in water temperature. Here, gonad development commenced in autumn when water temperature dropped below 20 ^{o}C, peaked in June when it was approximately 12 ^{o}C, and slowed as temperature rose through to October. For the Tasmanian population, despite a slightly longer period of gonad development, development occurred over a similar range of temperatures. Kurth (1957) indicated that mean oocyte diameter in the ovaries of developing fish began to increase in February of each year, as water temperature fell below 20 ^{o}C and was highest between April and September when water temperature was <12 ^{o}C. As such, the slightly longer reproductive period for populations in Tasmania due to earlier onset and later recession of gonad development (Kurth 1957) was likely related to different water temperature regimes between the two regions. Whilst this provides strong evidence for a possible causal relationship between gonad development and water temperature, other indirect factors such as photoperiod may also influence gonad development for *R. tapirina* (Pankhurst and Porter 2003; Pankhurst and Fitzgibbon 2006).

4.4.2   *Reproductive mode*

The occurrence of oocytes at different stages of development can be indicative of the reproductive mode for a species of fish (Fowler et al. 1999). Ovaries of *R. tapirina* exhibited features associated with indeterminate fecundity. These were the co-occurrence of oocytes at different stages of development, including POFs, and a continuous size distribution of oocytes with no gaps between developmental stages, except that between advanced yolked and hydrated oocytes. This suggests that oocytes developed from the unyolked stage to the hydrated stage continually during the reproductive season. As such, the number of oocytes released throughout a season could not be determined at the beginning of the spawning season, and so fecundity was indeterminate (Hunter and Macewiecz 1985).

The size distribution of oocytes at different developmental stages also indicated that not all advanced oocytes became hydrated and were released on one occasion. Rather, numerous batches of advanced oocytes developed and became hydrated during the reproductive season. This, along with the simultaneous presence of oocytes at different stages of development suggests that oocyte development was asynchronous (West 1990). Moreover, the co-occurrence of hydrated oocytes and post-ovulatory follicles in some ovaries indicated that spawning by individual fish occurred on more than one occasion. These features indicate that *R. tapirina* is a multiple batch spawner

with asynchronous oocyte development and indeterminate fecundity. These characteristics are consistent with those of the Tasmanian populations (Kurth 1957; Barnett and Pankhurst 1999).

The reproductive mode described here for *R. tapirina* is characteristic of numerous other temperate flatfish species (Manning and Crim 1998; Nagler et al. 1999; Rijnsdorp and Witthames 2005). Multiple batch spawning is considered a bet-hedging strategy to deal with uncertain environmental conditions for survivorship of offspring (Wilbur and Rudolf 2006). Such a strategy is considered particularly important for estuarine fish species, such as *R. tapirina*, as it reduces the risk of recruitment failure by spreading reproductive effort over time and enhances the likelihood that at least some progeny will experience favourable conditions for growth and survival at least some time through the reproductive season (Nakayama et al. 2011).

4.4.3   *Spawning activity*

Microscopic analysis of ovaries was done to provide a more accurate indication of when spawning occurred in the two areas of the Coorong estuary. Estimates of spawning fraction for both areas indicated that spawning activity followed a similar seasonal pattern to that of gonad development, i.e. the proportion of spawning females was low in March, increased in April, remained high to September and subsequently declined. For the Estuary, the peak spawning fraction of 0.71 in May indicated that, on average, females were spawning once every 1.4 days at this time. In comparison, females in the North Coorong were spawning every second day. Unfortunately, restricted sampling limited the capacity to determine the period over which this high spawning frequency was sustained.

Despite the contrasting salinity regimes of the two study areas, there were strong similarities in spawning activity between the Estuary and North Coorong. For each month in 2010, there was no difference in spawning fraction between areas. The one exception in August, when a higher proportion of spawning females were detected in the Estuary, was likely a consequence of the small number of females sampled during that month, rather than differences in the environmental and/or physiological processes that influence spawning. The similarity in spawning activity between the Estuary and North Coorong suggests that either mixing occurred between the two areas, or that differences in salinity did not affect the physiological processes that first lead to gonad development and then to the maturation of oocytes.

The lack of variation in spawning activity between near the Murray Mouth and up to 25 km away in North Coorong indicated that spawning was not limited to a specific area in the Coorong

estuary. In Tasmania, *R. tapirina* commonly spawned near the mouths of estuaries, while some individuals migrated to deeper offshore habitats to spawn (Kurth 1957; Crawford 1984). However, conditions in Tasmanian estuaries would have been vastly different to those in the Coorong estuary in 2009 and 2010, as fresh conditions would have occurred in the upper reaches and often at the mouths of estuaries during periods of high freshwater inflow. In the present study, the lack of freshwater inflows to the estuary resulted in a large contraction in the habitat available to *R. tapirina.* While this limited spawning to within 25 km of the Murray Mouth, it is not known whether any adults moved offshore due to the poor environmental conditions. Spawning migrations from estuaries to offshore grounds is common for several other flatfish species including *Solea solea* (Koutsikopoulos et al. 1995)*, Pleuronectes platessa* and *Atheresthes stomias* (Minami and Tanaka 1992; Rickey 1995; Gibson 1997).

Whilst water temperature and gonad development were correlated, there was evidence for a possible causal relationship with spawning activity for *R. tapirina.* In both study areas, spawning commenced in autumn when the water temperature was approximately 20 ^{o}C, it was most frequent in winter when water temperatures were between 11 ^{o}C and 14 ^{o}C, and it ceased when temperature exceeded 19 ^{o}C in late spring. These results differ from those for Tasmanian populations, which spawned only when water temperatures were less than 13 ^{o}C (Kurth 1957). Differences between the two populations suggest that environmental factors, other than temperature, may influence spawning activity for this species. Photoperiod, i.e. day length, is regarded a primary determinant of the phasing of spawning for numerous temperate fish species (Pankhurst and Porter 2003). Pankhurst and Fitzgibbon (2006) showed that spawning by captive *R. tapirina* was not tightly regulated by photoperiod, but suggested that a temperature-photoperiod interaction is likely to affect spawning activity, particularly for wild populations. Other factors including poor water quality and reduced food availability are also known to limit spawning activity (Hilder and Pankhurst 2003; Bayarri et al. 2004; Guèye et al. 2013).

4.4.4   Sexual maturity

Sexual maturity represents a critical phase in the life of fish when allocation of energy shifts from growth and survival to include reproduction (Trippel and Harvey 1991; Froese and Binohlan 2000). For *R. tapirina*, males attained sexual maturity at a larger size than females. These findings conflict with those from Tasmania, where males matured at a smaller size than females (Crawford 1984). While such differences are not unexpected given the geographic separation of the two populations (Stergiou 1999), differences should be interpreted cautiously given the low number of

males available to this study. Nonetheless, they provide the first evidence that both male and female *R. tapirina* are likely to spawn in their first winter.

4.4.5   Fecundity

Estimates of annual egg production were necessary to establish the reproductive potential of *R. tapirina*. Here, *R. tapirina* was found to be a highly fecund species that released multiple batches of eggs over a protracted spawning season. Despite the likely under-estimation of batch size resulting from counts of hydrated oocytes only, e.g. a subset of the annual stock of oocytes (Jakobsen et al. 2009), the positive linear relationship between batch size and fish length for *R. tapirina* indicated that the reproductive potential of the South Australian population is greatly increased by the presence of larger individuals. Nonetheless, ongoing monitoring of the fine-scale temporal variation in the frequency of spawning events is required to accurately determine the reproductive output of *R. tapirina* populations in the Coorong estuary.

This study contributed important information on the reproductive biology and spawning dynamics of *R. tapirina.* The reproductive strategy of this species involving the release of multiple batches of eggs over a prolonged period during the cooler months of each year, likely enhances the resilience of Coorong populations during periods of poor environmental conditions by increasing the likelihood that at least some offspring will experience conditions favourable for growth and survival. Strong spatial similarities in spawning activity and fecundity between two areas characterised by different salinity regimes suggests that either mixing occurred between the two areas, and/or the processes associated with the production, maturation and spawning of oocytes was not directly affected by the different salinity regimes. The previously–documented variation in population size for *R. tapirina* is likely a consequence of environmentally-mediated processes that influence egg production and recruitment success.

5   Variation in the diet of greenback flounder (*Rhombosolea tapirina*) affected by freshwater inflows in the Coorong estuary

5.1   Introduction

Estuaries often support high densities of fish through the provision of refuge from predators (Haedrich 1983; Lankford and Targett 1994), different water temperature regimes (Beck et al. 2001), and the rich supply of invertebrate prey, which constitutes an important part of the food for fishes (Hyndes et al. 1997; Hadwen et al. 2007). However, prey abundance can vary spatially and in relation to environmental changes (Bottom and Jones 1990; Edgar and Shaw 1995b; Griffiths 1997), which is likely to affect the carrying capacity of the estuary for fish populations (Feyrer et al. 2007). Recruitment, growth and maturation of fish depend directly or indirectly on the availability of food resources (Wootton 1990). As such, knowledge of the feeding biology of estuarine fish species and how feeding may be affected by environmental changes will help to better understand their population dynamics, which is of fundamental importance to the management of fisheries.

Estuarine fishes are trophically diverse, displaying a diversity of feeding habits and modes that generally reflect their adaptations to exploit the food resources of the habitats they occupy (Elliott et al. 2002). The main division of feeding modes is between generalists, which are species with a diverse diet and broad dietary niche, and specialists, such as predators that have low prey diversity and narrow niche width (Amundsen et al. 1996). While most estuarine fishes are generalists (Hostens and Mees 1999; Baldoa and Drake 2002; Elliott et al. 2002), feeding mode can change throughout an individual's life, reflecting the development of its feeding apparatus (Scharf et al. 2000), sensory ability (Batty and Hoyt 1995) and swimming capabilities (Gibson 2005). Feeding can also change over much finer temporal scales, such as seasonally and/or diurnally (Howell et al. 2004; Earl et al. 2011). Therefore, understanding the feeding ecology of a species must consider how its feeding habits change with fish size and over time.

The traditional approach to studying fish feeding involves gut contents analysis. If prey items in the gut are easily identified, this technique is very effective and can provide measures of the volumetric contribution of different food sources to the diet (Hyslop 1980). However, this

approach has its limitations as it only provides a snapshot of the diet based on what was consumed in the hours prior to capture and can provide biased results relating to material that is identifiable in the gut, but may not actually be assimilated (Cocheret de la Moriniere et al. 2003; Fanelli et al. 2011). More recently, the analysis of naturally occurring stable isotopes of carbon (δ^{13}C) and nitrogen (δ^{15}N) in the tissues of consumers and their prey has been used to examine trophic relationships among organisms (Peterson and Fry 1987). Stable isotope signatures can provide insight into the long-term feeding behaviour of consumers, because they integrate the feeding history over longer periods of time, i.e. months (Peterson 1999). Thus, when used in combination with gut content analysis, stable isotopes analysis can be a useful complementary tool for assessing trophic relationships between consumers and their prey.

Flatfish are renowned predators in estuaries worldwide (Livingston 1987; Link et al. 2005). Their flattened body and camouflaged, binocular topside facilitates a strong trophic interaction with a broad range of prey including benthic invertebrates, fish and squid (Holmes and Gibson 1983; Livingston 1987; de Goeij et al. 2001; Link et al. 2002; Link et al. 2005; Gibson 2005). The greenback flounder, *Rhombosolea tapirina*, is the most common flatfish species in estuaries and inshore coastal waters of southern Australia and New Zealand (Gomon et al. 2008; Froese and Pauly 2013). The diet of larval and small juvenile *R. tapirina* was described several decades ago (Crawford 1984; Jenkins 1987b; Shaw and Jenkins 1992), yet no information exists on the feeding habits of larger juvenile and adult *R. tapirina.*

Based on commercial fishery catch records, *R. tapirina* have been highly abundant in the Coorong estuary (Ferguson 2007), which has been subject to extreme environmental changes in recent years (Kingsford et al. 2011). During the 2000s, the Coorong estuary underwent significant ecological change mainly due to severe drought in the Murray-Darling Basin (Leblanc et al. 2012). The lack of freshwater inflow to the estuary during this period drastically increased salinities throughout the system, affecting the distribution and abundance of *R. tapirina* (Chapter 2) and macroinvertebrate communities (Dittmann et al. 2010), which likely constitute an important part of the diet for *R. tapirina.* Since September 2010, there has been a large, continuous freshwater release into the Coorong, which resulted in a rapid decrease in salinity throughout the system and a subsequent range extension and increase in abundance of *R. tapirina* (Chapter 2). While high inter-annual variation in population abundance is characteristic of this population (Ferguson 2007), it is unknown to what extent its feeding habits and requirements may have influenced the changes in distribution and abundance.

This study described the dietary composition of *R. tapirina* in the Coorong estuary to provide a better understanding of its feeding habits and how these may have been affected by recent changes in the environment. The specific objectives were to: (1) determine the dietary composition of three different size classes of *R. tapirina*; (2) to compare the diets between two areas of different salinity regimes; (3) to assess whether there is evidence for seasonal variation in feeding pattern; and (4) to explore the link between dietary composition and environmental variables including water temperature and salinity. These objectives were investigated with gut content analyses, and the trophic position and likely assimilatory contribution of key prey items to the diet was assessed using stable isotope analysis (referred to as SIA hereafter).

5.2   Materials and methods

5.2.1   Study area

The study was undertaken in the Coorong estuary, South Australia (Figure 5.1). This estuarine system is a long (~110 km), narrow lagoon that extends south-east from the river mouth and represents one of the largest estuarine habitats in Australia. The original Murray River estuary covered 660 km^2 and included the brackish Lakes Alexandrina and Albert and the Coorong lagoons. In the early1940s, a series of barrages were constructed between the Lakes and the Coorong, reducing the estuary to 11% of its original size (Leblanc et al. 2012). Since then, water extraction for agriculture and human consumption has reduced mean annual flow to 40 % of natural levels (Kingsford et al. 2011). The regulation of remaining river flows has led to inconsistent freshwater inflow to the Coorong estuary (Figure 5.2), including prolonged periods of no flow, which had serious consequences for the abundance and distribution of numerous finfish species that use the estuary (Ye et al. 2013).

5.2.2   Environmental parameters

Daily estimates of mean electrical conductivity for the period 1st December 2009 to 30th November 2010 were obtained from continuous water monitoring stations at two locations in the Coorong estuary (Figure 5.1) by the Department of Environment, Water and Natural Resources (Government of South Australia 2013). Daily mean electrical conductivity was used as a proxy for salinity and is referred to as salinity hereafter (freshwater = 0 µS/cm; seawater ~ 50 000 µS/cm). Mean daily water temperature was recorded at each station using independent temperature data loggers (HOBO® Pendant Temperature Data Logger). Loggers were attached to a beacon approximately one metre below the low tide mark and recorded water temperature every

2 hours for the duration of the study period. On each sampling occasion, oxygen saturation was also recorded using a hand-held YSI 85 electrode.
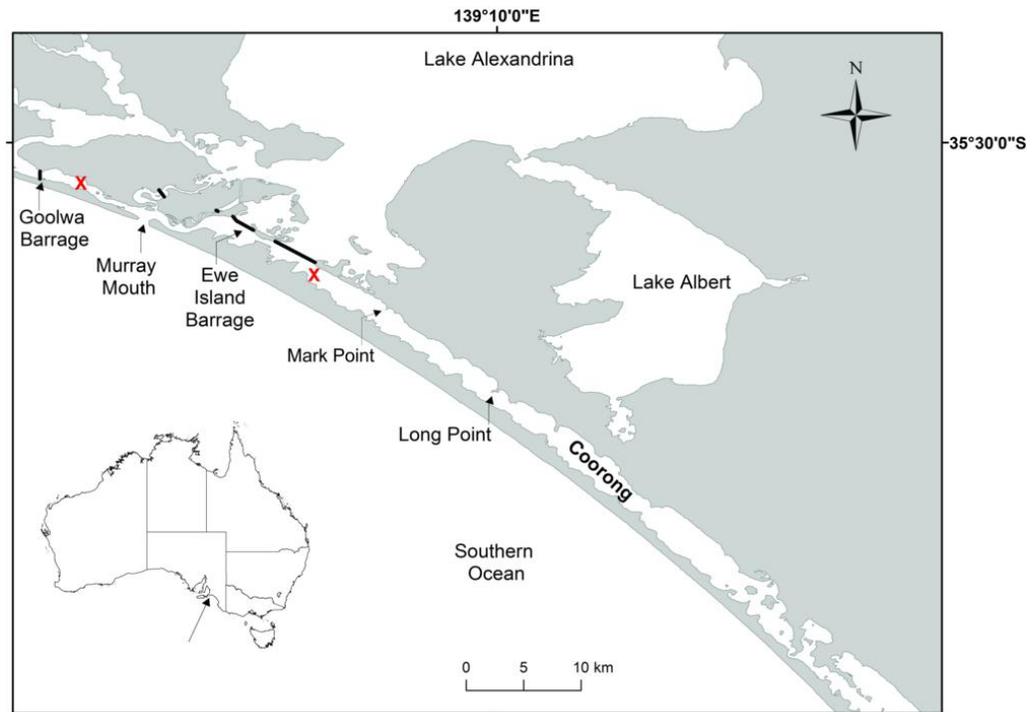
Figure 5.1 Map of the Coorong estuary showing the location of the barrages that separate the estuary from Lake Alexandrina (thick black lines). Navigational beacons (X) on which the temperature data loggers were deployed are also shown.
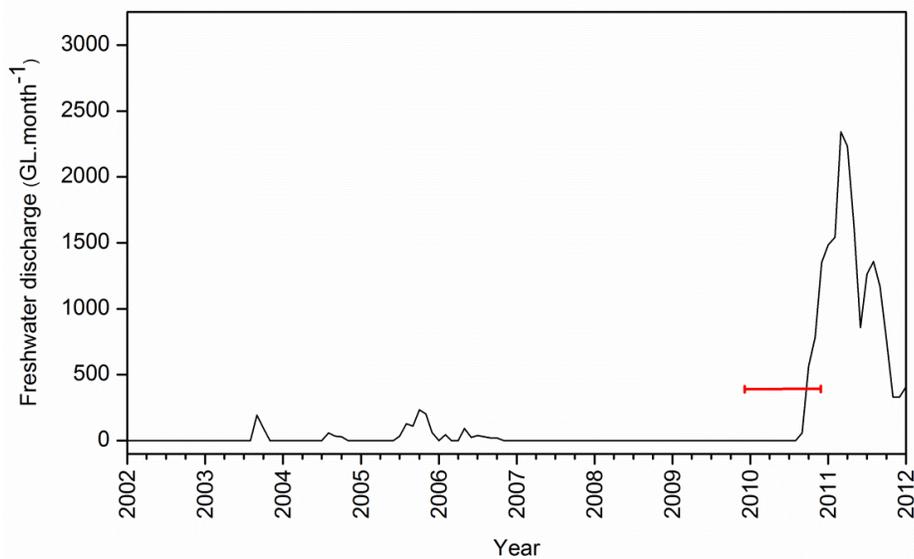
Figure 5.2 Estimates of total monthly freshwater discharge through the barrages and into the Coorong estuary from 1st January 2002 to 1st January 2012 (data source: Murray-Darling Basin Authority 2013). Capped red line shows timing of the study period relative to discharge.

5.2.3   Sample collection and processing

In total, 475 juvenile and adult *Rhombosolea tapirina* were collected between December 2009 and November 2010 from two study areas. One study area, the Estuary, was located near the Murray Mouth and included all waters downstream of the Goolwa Barrage to the Ewe Island Barrage (Figure 5.1). The other, North Coorong, extended from the Ewe Island Barrage to Long Point. Fish were collected from the commercial fishing sector, which targets *R. tapirina* with 4.75″ mesh gill nets. Such nets are size-selective and rarely capture fish < 220 mm and > 360 mm total length (TL). Scientific sampling was also done using a 3.5″ mesh haul net and a 1″ mesh seine net to capture a broader size range of fish, including those < 220 mm TL. Each fish was measured for total length (TL) and separated into one of three size classes, i.e. small: ≤150 mm TL; medium: 151-250 mm TL; or large: ≥251 mm TL, and immersed into an icy brine as soon as possible after capture. Sampling effort and catch rates varied considerably throughout the study period, though fish were collected in most months of each season (Table 5.1).

Table 5.1 Summary of the samples collected from the Estuary and North Coorong during the study period. Method types: H – haul net; S – beach seine net; G – commercial gill net. * Samples used for SIA.

Season	Sample date	Estuary			North Coorong		
		Number of samples	Sample size	Method	Number of samples	Sample size	Method
Summer	Dec-09	1	9	H	2	21	H,S
	Jan-10	1	23	H,S	-	-	-
	Feb-10	2	20*	G,S	2	17*	H,S
Autumn	Mar-10	2	20	H,S	2	23	H,S
	Apr-10	2	16	G,S	2	16	G,S
	May-10	3	43	G,H,S	2	29	G,H
Winter	Jun-10	1	24	H	1	15	G
	Jul-10	2	22	H,S	1	18	G
	Aug-10	1	13	G	2	12	H,S
Spring	Sep-10	1	15	H,S	-	-	-
	Oct-10	2	36	G, S	1	14	H
	Nov-10	2	29*	G,H,S	4	40*	H,S
	Total	20	270		19	205	

Fish were dissected within 8 hours of collection. Each fish was re-measured for TL to the nearest millimetre and weighed to the nearest 0.01 g. For gut contents analysis, the gut was removed and placed into a sample jar containing 10 % formalin/seawater solution. For fish samples collected in February (summer) and November (spring) 2010, five individuals from each size class were

randomly selected, and a small portion (~1 g wet weight) of white dorsal muscle tissue was removed for stable isotope analysis (Table 5.1). The muscle tissue of each fish was rinsed in Milli-Q water, placed in a glass vial and kept frozen at -20°C until further processing.

5.2.4   Gut contents analysis

Prior to analysis, the preserved gut was removed from the formalin solution and placed under running water to remove excess formalin. The contents of the gut were removed and placed in a Petri dish for sorting under a dissecting microscope. Food items were sorted into taxonomic categories. Material in each category was spread to a consistent thickness over laminated graph paper and the volume was calculated by multiplying its area by the depth to which it was spread (Earl et al. 2011).

Fish with empty stomachs were not considered for data analyses. Several indices were used to describe diet composition and determine variation in diet. The Occurrence Index (O_i) is defined as the percentage of fish sampled at a given time that contained a particular prey item (Hyslop 1980) and was calculated to show the relative contribution of different prey categories. The relative volumetric contribution (V_i) of each prey category to the total volume of food in each gut was also determined. The Index of Preponderance (IP), which considered estimates of O_i and V_i, was used to rank the different prey categories in order of importance based on their contribution to the diet (Marshall and Elliot 1997). It was calculated using the equation: $IP = (V_iO_i)/\sum(V_iO_i)$. The Shannon-Wiener diversity index ($H' = -\Sigma p_i Log_e p_i$) (Pielou 1974) was used as a measure of the diversity of the diet, where p_i was the proportion of the total volume of food accounted for by the different prey categories. Shannon-Wiener indices were calculated for each size class from each area.

5.2.5   Stable isotope analysis (SIA)

Food source collection

SIA relies on the matching of elemental signatures of prey material to those in the muscle tissue of the consumer (Peterson and Fry 1987; Peterson 1999). Potential invertebrate prey items were collected from the two study areas in February (summer) and November (spring) 2010 using a variety of sampling methods. Benthic invertebrates were collected from the intertidal and subtidal zones of each area using a hand-held Ekman grab (225 cm^2 surface area), PVC corers and a 500 μm sieve, while larger invertebrates were collected using an 8-m seine net (mesh size 4 mm). Several horizontal plankton net tows (50 cm net diameter, net mesh size 500 μm, cod-end mesh

size 300 μm) were performed at the surface of the main channel to collect zooplankton. In each study area, plankton net tows done in February 2010 contained mostly gammarids, while those collected in December also contained copepods and mysid shrimp. Where possible, three samples of ~1 g (wet weight) for each potential prey item were collected, placed in labelled zip-lock bags and stored on ice until further processing in the laboratory. Where possible, invertebrates were kept alive for up to 24 hours so that ingested material could be excreted before processing (Paterson and Whitfield 1997).

In the laboratory, all potential prey items were sorted to the lowest possible taxonomic category. Shells of molluscs and the exoskeletons of larger crustaceans were removed to ensure they did not influence isotopic values (Hadwen et al. 2007). Material for each prey category was rinsed with Milli-Q water and stored frozen at -20°C until further processing.

Whilst the main dietary items, based on the results of gut content analysis, were collected in sufficient amounts for SIA in summer, numerous other prey taxa identified in the guts of *R. tapirina* were not obtained by sampling. In spring, only chironomid larvae, copepods, mysids and gammarids were collected in numbers sufficient for analysis, presumably due to the abrupt change in salinity associated with high freshwater discharge to the system (Dittmann et al. 2011a).

Preparation for analysis

All frozen invertebrate and fish muscle samples were placed in a -80°C freeze dryer (VirTis BenchTop) with a vacuum applied (~30 mTorr) until lyophilisation was complete. Once dried, each sample was ground into a fine powder using a mortar and pestle. For each sample, a sub-sample of ~600 μg and ~200 μg for nitrogen and carbon, respectively, were placed into separate 8 mm tin-capsules. The contents of each capsule were analysed for δ^{13}C and δ^{15}N using a continuous-flow isotope ratio mass spectrometer (GV Instruments IsoPrime EA/IRMS) at Flinders Advanced Analytical Laboratory, Flinders University. Standards against which material were assessed were sucrose for δ^{13}C and urea for δ^{15}N. Stable isotopic values were expressed in parts per thousand (‰), as deviations from standards according to the formula: δ^{13}C or δ^{15}N = $[(R_{sample}/ R_{standard}) - 1] \times 10^3$, where R is ^{13}C/^{12}C or ^{15}N/^{14}N. The isotopic analyser was calibrated regularly to minimise error in the weighing procedure.

5.2.6   Data analysis

Gut contents analysis

Estimates of the volumetric contribution of each category of prey items to the diet of individual fish were considered to identify how feeding habits varied among study areas, size classes and seasons. Prior to analysis, dietary data were square root transformed, and Bray-Curtis similarities were calculated to produce a similarity matrix. Principle Coordinate plots (PCO) were used to display differences in diet composition across size classes and seasons for each study area. Differences in the volumetric-based composition of the diet were then identified using a three-way permutational analysis of variance (PERMANOVA) with area (2 levels), size class (3 levels) and season (4 levels) as fixed factors (α=0.05). Where a significant effect was detected, pair-wise analyses were used to determine which levels were statistically different (Anderson et al. 2008). A similarity of percentages (SIMPER) analysis was undertaken to identify categories of prey taxa that contributed most to the dissimilarity between groups. A 60 % cumulative contribution cut-off was applied.

Non-abundant prey taxa in the diet of *R. tapirina* may 'characterise' the diet without largely contributing to the differences between groups as detected by SIMPER. Such species may be important indicators of the differences in diet among groups. As such, indicator species analysis (Dufrêne and Legendre 1997) was used to calculate an indicator value for each prey category based on its group fidelity and relative volumetric contribution to the diet. The statistical significance of the indicator value was determined for each category of prey taxa in each group using the Monte Carlo (randomisation) technique ($\alpha = 0.05$). Indicator species analysis was undertaken using the package PC-ORD v 5.12 (McCune and Mefford 2005).

To explore the relationship between dietary composition and environmental variables in each study area, distanced-based multiple regression linear models (DISTLM) were calculated using forward selection of the environmental variables and visualised using distanced-based redundancy analyses (dbRDA) (Anderson et al. 2008). This method generated a prediction of the relative influence of water temperature, salinity and oxygen concentration on determining differences in diet between size classes and seasons. Vector overlays were used on the dbRDA plots to indicate the relative strength of their correlation (Spearman rank) with groups of samples.

<u>Stable isotope analysis</u>

SIA was used to assess the relative importance of the prey items to the diet for each size class of *R. tapirina* from each study in summer and spring. However, this was limited by the relatively low number of potential food sources available. Nonetheless, isotopic signatures of *R. tapirina* and potential prey items were represented graphically and interpreted based on the overlap of error bars of signatures of *R. tapirina* and prey, and with respect to the baseline signature for δ^{15}N for the primary consumers (i.e. reflecting the second trophic level in the food web). Baseline signatures for each area were estimated by taking the average value of δ^{15}N from all primary consumers sampled. As there is only a minor enrichment of < 1‰ of δ^{13}C from prey to the consumer, ratios of δ^{13}C in tissues of consumers generally reflect the isotopic composition of their diet (McCutchan et al. 2003). An enrichment value of 3.4‰ was used to account for the fractionation increase of δ^{15}N (Post 2002; McCutchan et al. 2003) and to determine trophic levels in the stable isotope plots. The trophic positions (TP) of the three size classes of *R. tapirina* from each area were calculated with the equation: $\text{TP}_{R.\ tapirina} = \lambda + (\delta^{15}\text{N}_{R.\ tapirina} - \delta^{15}\text{N}_{\text{baseline}})/ \Delta n$, where λ is the trophic position of the baseline (i.e. primary consumers = 2) and Δn is the trophic enrichment mean of 3.4‰ (Post 2002).

Data on trophic position for *R. tapirina* were compared between areas (2 levels), size classes (3 levels) and season (2 levels, i.e. summer and spring) using a three-factor PERMANOVA performed on a Bray-Curtis similarity matrix of untransformed data. Pairwise comparisons were used to compare trophic positions among size classes. All statistical analyses, except for indicator species analysis, were performed using the statistical software package PRIMER v. 6. 1. 15 and PERMANOVA + (Anderson et al. 2008).

5.3   Results

5.3.1   *Environmental characteristics*

In the two study areas, there was distinct seasonality in water temperature. In the Estuary, water temperature was highest in January and lowest in July (Figure 5.3). In North Coorong, water temperature conformed to the same seasonal pattern as observed for the Estuary, but was more variable (Figure 5.3). Salinity in the Estuary was relatively stable from December 2009 to August 2010 and averaged 55,251 µS/cm.day^{-1} (range: 44,248 - 61,087 µS/cm.day^{-1}) (Figure 5.3). In September 2010, the recommencement of continuous freshwater inflows reduced salinity to < 8,000 µS/cm.day^{-1}, where it remained until late November. In North Coorong, salinity was

generally higher and more variable compared to the Estuary, averaging 61,513 µS/cm.day^{-1} (range: 49,973 µS/cm.day^{-1} – 99,960 µS/cm.day^{-1}). The decrease in salinity associated with the commencement of flows occurred later in North Coorong compared to the Estuary, i.e. mid-September 2010, when it decreased to < 20,000 µS/cm.day^{-1} and remained low until at least the end of November. Oxygen saturation followed a similar temporal trend as salinity in the two study areas, i.e. it was relatively high from December 2009 to August 2010 and declined rapidly with the commencement of freshwater inflows in September 2010 (Figure 5.3).
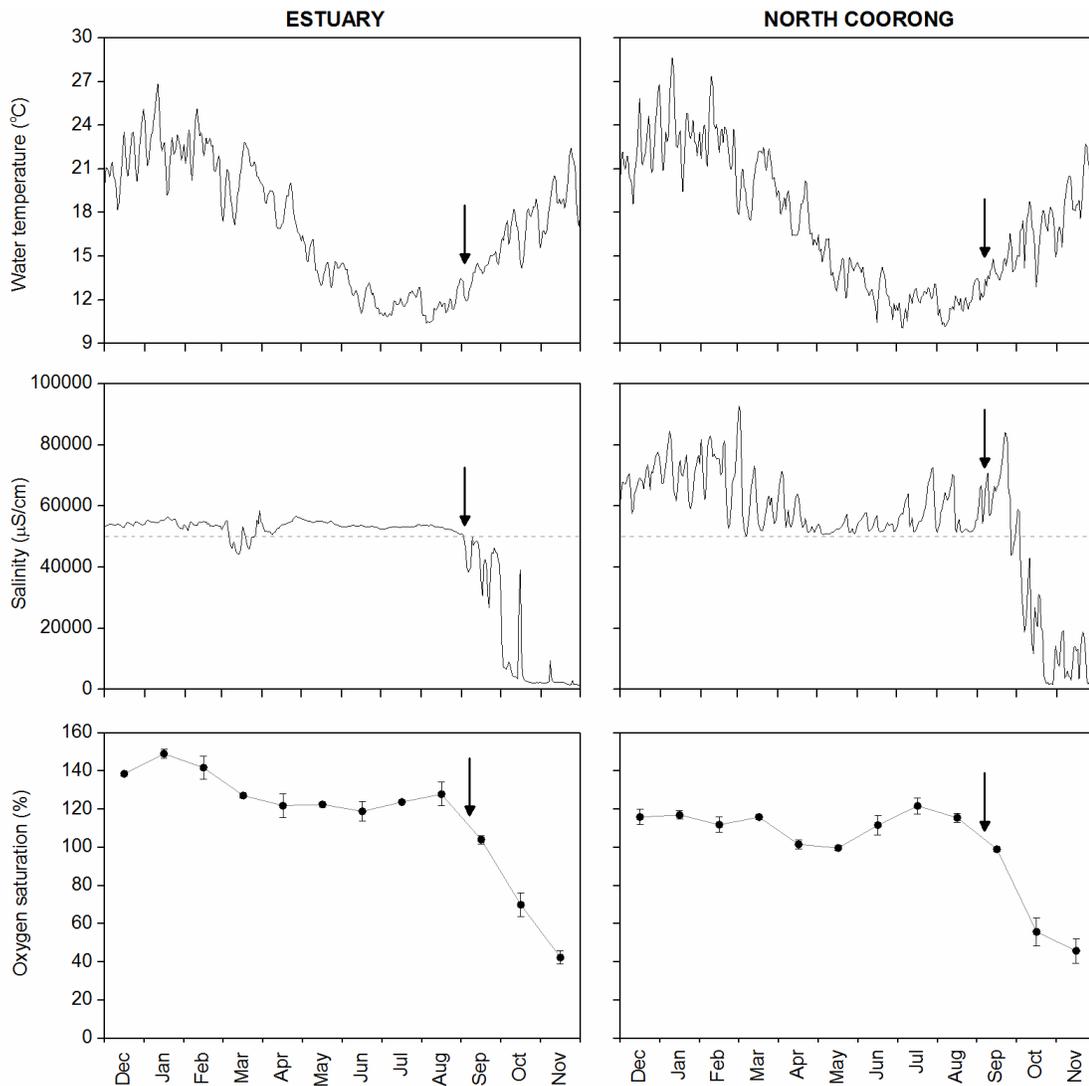
Figure 5.3 Mean daily water temperature, salinity and oxygen saturation for the Estuary (left) and North Coorong (right) from 1st December 2009 to 30th November 2010. Broken grey lines represent the approximate salinity of seawater (50,000 µS/cm). Black arrows indicate the time freshwater flow commenced.

5.3.2   Sample characteristics

In total, the guts of 475 *R. tapirina* were considered for dietary analysis, of which 398 (85 %) contained prey items. Fish sampled from the Estuary (*n*=233) and North Coorong (*n*=165) ranged in size from 23 to 357 mm TL and 34 to 349 mm TL, respectively (Figure 5.4).
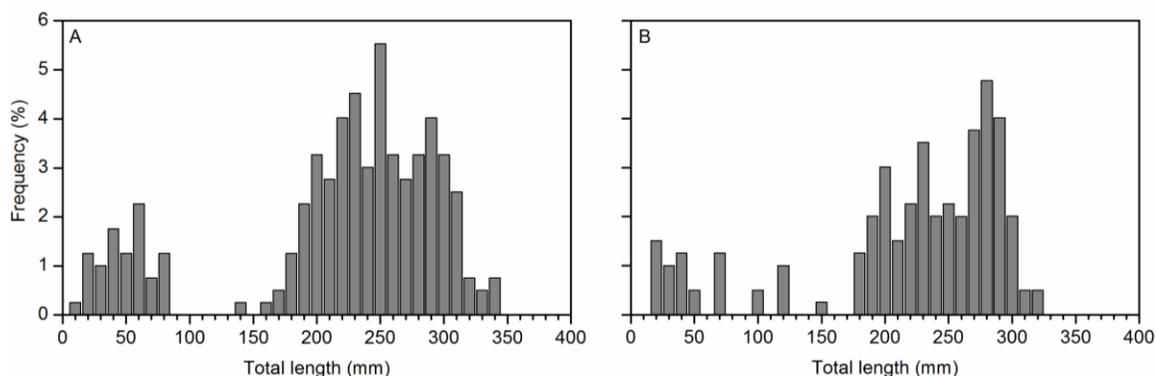
Figure 5.4 Size frequency distributions of *R. tapirina* considered for the analysis of the diet from (A) Estuary (*n* = 233); and (B) North Coorong (*n* = 165).

5.3.3   Gut content analyses

Overall, 28 different prey categories were represented in the diet of *R. tapirina* which included annelids, crustaceans, molluscs, insects and fish (Table 5.2). All prey items in the guts had been severely masticated, yet most could be classified to at least a broad prey category. Pharyngeal mucus encased some prey items which complicated their identification, but overall there were relatively few unidentifiable items.

There was considerable variation in the Indices of Preponderance (IP) for the different categories of prey items among size classes within and between the two study areas (Table 5.2). Overall, gammarid amphipods (referred to as gammarids hereafter) and the polychaete *Nephtys australiensis* were the most dominant prey items consumed, while the siphons of bivalves were also consumed in relatively high volumes (Table 5.2). Collectively, these three prey items accounted for > 90 % of the diets in the two study areas. The Shannon-Wiener Indices for each area indicated a greater diversity of prey categories in the guts of the medium and large size classes than the small size class (Figure 5.5). In each study area, the diets of the small size class were dominated by small crustaceans including gammarids, mysids and copepods, while lesser contributions were made by chironomid larvae and bivalve siphons. The diets of the medium and larger size classes consisted mainly of *N. australiensis,* gammarids and the bivalve siphons (Table

Table 5.2 Summary of the occurrence index (O_i), mean volumetric contributions (V_i), and Index of Preponderance (IP) for the three size classes of *R. tapirina* for the Estuary and North Coorong. Sample sizes for each size class are shown in brackets.

| | Estuary | | | | | | | | | North Coorong | | | | | | | | |
| | ≤ 150 mm (42) | | | 151-250 mm (87) | | | ≥ 251 mm (106) | | | ≤ 150 mm (26) | | | 151-250 mm (63) | | | ≥ 251 mm (74) | | |
Prey taxa	O_i	V_i	IP	O_i	V_i	IP	O_i	V_i	IP	O_i	V_i	IP	O_i	V_i	IP	O_i	V_i	IP
Annelida																		
Nephtys australiensis	16.7	5.5	1.33	80.5	34.1	48.81	73.6	43.1	56.27	7.7	1.6	0.18	69.8	33.8	44.6	62.2	31.9	42.7
Phyllodoce novaehollandiae				9.2	1.8	0.29	6.6	0.7	0.08							6.8	0.2	0.0
Simplisetia aequisetis				1.1	0.7	0.01	4.7	0.1	0.00				1.6	0.4	0.0	6.8	1.5	0.2
Ficopomatus enigmaticus	2.4	0.0	0.0	9.2	0.3	0.05	17.9	0.4	0.12				12.7	0.4	0.1	12.2	0.4	0.1
Capitellidae				1.1	0.1	0.00	4.7	0.2	0.02	11.5	2.5	0.44	1.6	0.1	0.0	6.8	0.1	0.0
Unidentified Polychaeta				16.1	5.5	1.57	21.7	5.7	2.21				6.3	2.2	0.3	6.8	2.9	0.4
Oligochaeta							0.9	0.0	0.00	11.5	2.2	0.39	1.6	0.2	0.0			
Crustacea																		
Paragrapsus gaimardii	2.4	0.1	0.00	5.7	1.4	0.14	19.8	3.79	1.33				7.9	1.4	0.2	21.6	3.6	1.7
Amphipoda: Gammaridea	97.6	48.9	69.31	70.1	25.1	31.29	67.9	26.1	31.52	96.2	51.9	77.20	55.6	23.4	24.5	58.1	18.1	22.7
Amphipoda (*Melita* sp.)				24.1	5.7	2.43	15.1	3.4	0.92	15.4	2.2	0.52	28.6	4.6	2.5	24.3	2.9	1.5
Amphipoda: other	16.7	1.3	0.31	1.1	0.2	0.00	1.9	0.0	0.00	11.5	1.9	0.35	1.6	0.1	0.0			
Melicertus latisulcatus	2.4	0.0	0.00	2.3	0.3	0.01	4.7	0.2	0.01							2.7	1.3	0.1
Mysidacea	28.6	7.0	2.89	2.3	0.3	0.01				26.9	4.0	1.68	3.2	0.0	0.0			
Biffarius sp.	2.4	0.1	0.00	3.4	0.2	0.01	13.2	1.9	0.45							2.7	0.8	0.0
Copepoda	54.8	15.3	12.14	1.1	0.0	0.00	1.9	0.0	0.00	38.5	6.7	3.97	1.6	0.0	0.0	2.7	0.2	0.0
Ostracoda	2.4	0.3	0.01	1.1	0.1	0.00	0.9	0.0	0.00									
Cumacea	4.8	0.5	0.04	2.3	0.4	0.02	2.8	0.1	0.01	38.5	4.7	2.81				1.4	0.0	0.0
Daphnia	2.4	0.3	0.01							11.5	2.9	0.51				5.4	0.1	0.0
Mollusca																		
Salinator fragillis							0.9	0.1	0.00									
Tellinidae (shell)				8.0	1.9	0.28	10.4	2.3	0.41				1.6	0.0	0.0	21.6	4.6	2.2
bivalve siphons	45.2	12.1	7.92	47.1	15.1	12.64	34.9	7.0	4.33	15.4	4.2	0.99	50.8	26.9	25.8	45.9	21.5	21.3
Notospisula sp.							0.9	0.0	0.00							2.7	0.1	0.0
Arthritica helmsi				2.3	0.2	0.01	3.8	0.0	0.00							1.4	0.0	0.0
Insecta (larvae)																		
Chironomidae	14.3	2.3	0.47	16.1	2.4	0.70	31.1	2.1	1.18	15.4	1.7	0.39	22.2	2.6	1.1	29.7	2.2	1.4
Dolichopodidae							2.8	0.1	0.00				3.2	0.5	0.0			
Ceratopogonidae							2.8	0.0	0.00									
Fish																		
Philypnodon grandiceps							1.9	0.2	0.01							1.4	0.0	0.0
Other																		
filamentous algae				5.7	0.3	0.03	11.3	0.2	0.05				9.5	2.3	0.4	40.5	6.4	5.6
unidentified material	59.5	6.4	5.53	24.1	4.0	1.70	27.4	2.2	1.05	50.0	13.7	10.57	19.0	1.1	0.4	1.4	1.2	0.0

5.2), although numerous other annelids, crustaceans, insect larvae and molluscs were also consumed but in relatively low quantities.
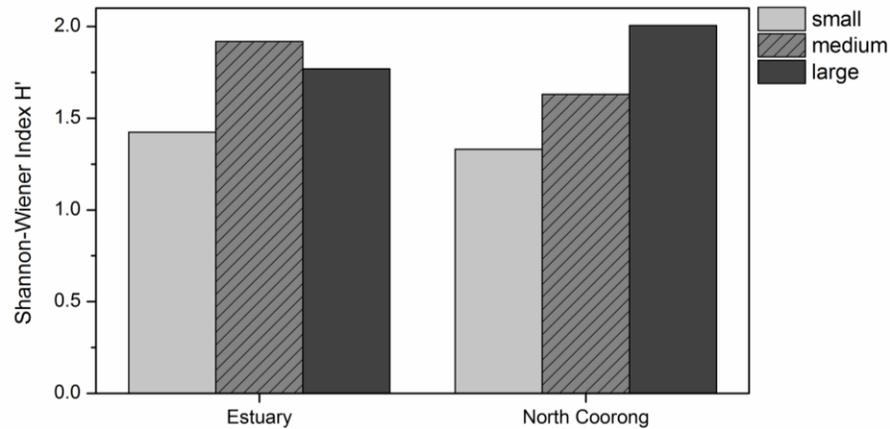
Figure 5.5 Comparative estimates of the Shannon-Wiener Index with respect to the diets of three size classes of *R. tapirina* sampled in the Estuary and North Coorong.

PCO plots showed distinct groupings of diets by season and size class for the two study areas (Figure 5.6). For each area, there was clear separation between the diets of the small size class and the two larger size classes, and between spring and all other seasons for the medium and large size classes. PERMANOVA indicated no significant effect of the interaction between area, size class and season on dietary composition, which was most likely due to the marginal effect of area (Table 5.3).

Table 5.3 PERMANOVA results for comparison of dietary composition of *R. tapirina* between area, size class and season in the Coorong estuary. P-values presented in bold are significant differences. ** = term has one or more empty cells.

Factor	d.f.	MS	Pseudo-*F*	P
Area	1	5016.7	2.69	**0.043**
Size class	2	19866	10.67	**<0.001**
Season	3	30708	16.49	**<0.001**
Area x Size class	2	35808	1.91	0.062
Size Class x Season	6	8248.6	4.43	**<0.001**
Area x Season	3	4177	2.24	**0.011**
Area x Size class x Season**	3	2068.6	1.11	0.337
Residuals	375	1861.8		
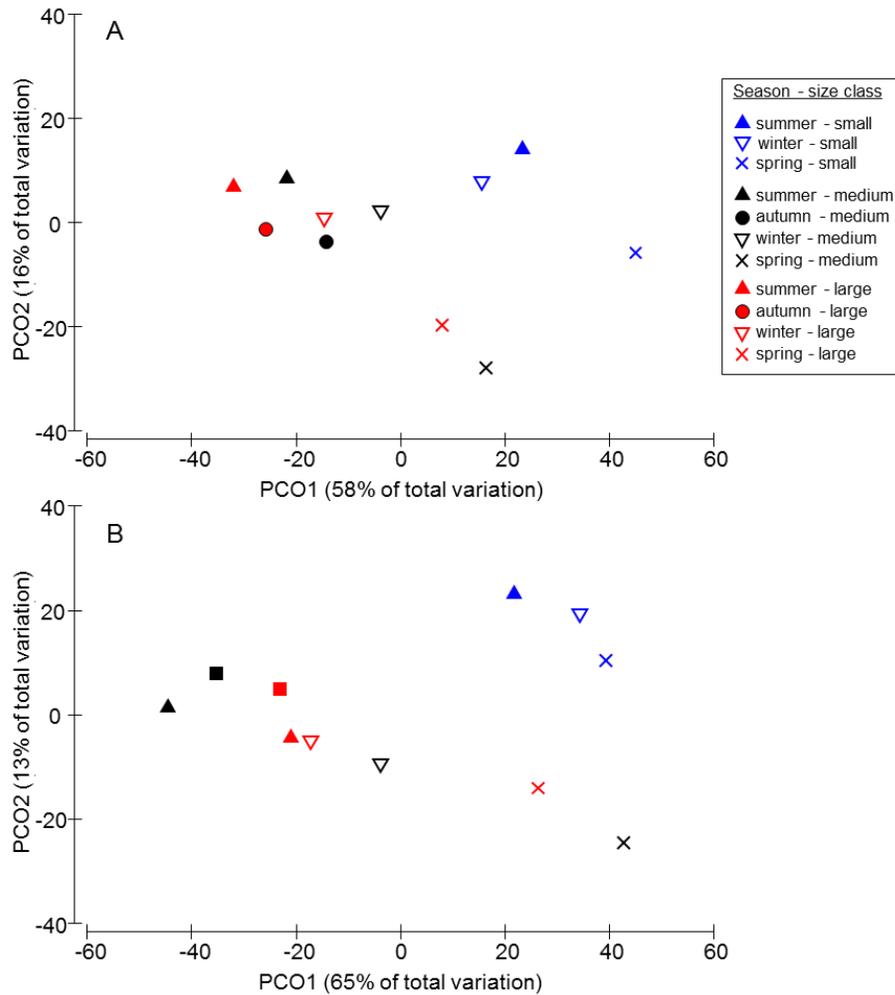
Figure 5.6 Principal coordinate analysis (PCO) plot of dietary composition (as mean percentage volume per group) of *R. tapirina* by season and size class for (A) the Estuary and (B) North Coorong. No fish samples were available for the small size class in winter from the Estuary and in summer and autumn from North Coorong.

Ontogenetic variation in diet

There was considerable variation in the contribution of the different prey items to the diet among size classes (Table 5.2). PERMANOVA indicated no interaction effect between area and size class on the diet of *R. tapirina*, indicating that differences between size classes were consistent among areas (Table 5.3). For each area, pair-wise tests detected significant differences in diet between the small size class and the medium and large size classes, though there were no such differences between the two larger size classes (Table 5.4). As such, data for the two larger size classes were pooled for subsequent analyses and are hereafter referred to as the medium/large size class.

Table 5.4 PERMANOVA pair-wise comparisons of diet composition between size classes of *R. tapirina* in the Estuary and North Coorong. Significant *P* values are highlighted in bold (α = 0.05).

Size class pairwise comparison	Estuary		North Coorong	
	t	P	t	P
small, large	5.5	**<0.001**	4.33	**<0.001**
small, medium	4.94	**<0.001**	4.5	**<0.001**
large, medium	1.29	0.154	1.18	0.215

For the Estuary, indicator species analysis showed that the diet of the small size class was characterised by small-bodied crustaceans including gammarids, copepods and mysids (Table 5.5). The diet of larger fish was characterised by larger invertebrates including *N. australiensis*, large amphipods (*Melita* sp.) and the shore crab *Paragrapsus gaimardii* (Table 5.5). Differences in diet between the small and medium/large size classes were driven mainly by the high contribution of gammarids, bivalve siphons and copepods in the diet of small fish and the higher contribution of *N. australiensis* in the diet of medium/large fish (Appendix 5.1; Figure 5.7).
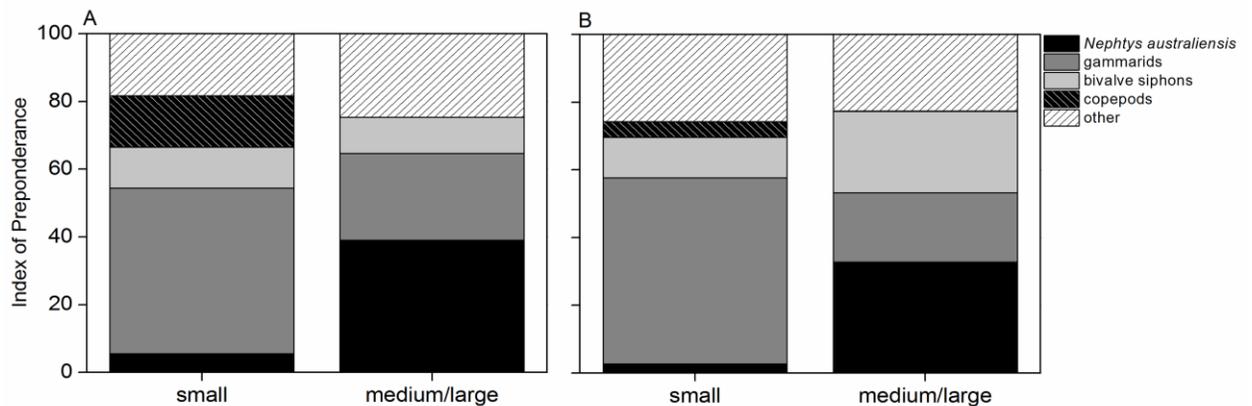
Figure 5.7 Comparisons of contributions of the different categories of prey taxa to the diet of *R. tapirina* between size classes for (A) the Estuary and (B) North Coorong based on the Index of Preponderance.

For North Coorong, the diet of the small size class was characterised by mainly small crustaceans, while the diets of medium/large fish were characterised by *N. australiensis* and bivalve siphons (Table 5.5). SIMPER analysis indicated that the relatively low contribution of gammarids and high contribution of *N. australiensis* and bivalve siphons to the diet of medium/large fish accounted for most of the dissimilarity between size classes (Appendix 5.1; Figure 5.7).

Table 5.5 Indicator species analysis of the size-related variation in dietary composition for the small and medium/large size classes of *R. tapirina* in the Coorong estuary. Data for the medium and large size class are grouped as indicated by PERMANOVA pair-wise comparisons. Only significant indicator species are shown (α = 0.05).

Study area	Prey taxa	Size class	Indicator value	P
Estuary	Amphipoda: Gammaridea	small	62.3	<0.001
	Copepoda	small	52.1	<0.001
	unidentified material	small	42.6	<0.001
	Mysidacea	small	27.8	<0.001
	Amphipoda: other	small	15.6	<0.001
	Nephtys australiensis	medium/large	63.8	<0.001
	Amphipoda (*Melita* sp.)	medium/large	19.2	0.014
	unidentified polychaete	medium/large	18.1	0.015
	Paragrapsus gaimardii	medium/large	12.1	0.05
North Coorong	Amphipoda: Gammaridea	small	68	<0.001
	Cumacea	small	38.3	<0.001
	Copepoda	small	37.3	<0.001
	unidentified material	small	37.1	<0.001
	Mysidacea	small	26.4	0.002
	Daphnia	small	11.5	0.003
	Amphipoda: other	small	11.2	0.004
	Oligochaeta	small	11	0.007
	Capitellidae	small	10.5	0.031
	Nephtys australiensis	medium/large	60.7	<0.001
	bivalve siphons	medium/large	38.9	0.015

Spatial variation in diet for each size class

While differences in diet between size classes occurred in both areas (Table 5.4), pair-wise comparisons detected marginal differences in diet between areas for the small (t = 1.212, P = 0.038) and medium/large size classes (t = 1.165, P = 0.041). For the small size class, copepods characterised the diet in the Estuary, while cumaceans and gammarids characterised the diet in North Coorong (Table 5.6). SIMPER analysis indicated that higher volumes of copepods and slightly lower volumes of gammarids consumed by the small size class in the Estuary contributed most to the dissimilarity between areas (Appendix 5.2; Figure 5.7).

For the medium/large size class, bivalve siphons were a significant indicator species for diets of larger fish in the Estuary, while large polychaetes and the ghost shrimp, *Biffarius* sp. characterised diets in North Coorong (Table 5.6). Spatial differences in diet were due mainly to lower contributions of *N. australiensis* and gammarids for North Coorong fish. These two items,

combined with bivalve siphons and large amphipods, accounted for 59 % of the dissimilarity

between areas (Appendix 5.2; Figure 5.7).

Table 5.6 Indicator species analysis of the spatial variation in dietary composition for the small and medium/large size classes of *R. tapirina*. Data for the medium and large size class are grouped as indicated by PERMANOVA pair-wise comparisons. Only significant indicator species are shown ($\alpha = 0.05$).

Size class	Prey taxa	Study area	Indicator value	P
small	Copepods	Estuary	33.7	0.024
	Cumacea	North Coorong	34.2	<0.001
	Amphipoda: Gammaridea	North Coorong	15.4	0.017
medium/large	bivalve siphons	Estuary	30.7	0.003
	Nephtys australiensis	North Coorong	41	0.047
	unidentified polychaete	North Coorong	12.1	0.012
	Biffarius sp.	North Coorong	7.1	0.02
	Phyllodoce novaehollandiae	North Coorong	6	0.05

Seasonal variation in diet for each size class

PERMANOVA detected a significant interaction between size class and season on diet

composition for *R. tapirina*, indicating that diet for each size class varied among seasons (Table

5.3). For each size class in the two study areas, pair-wise tests indicated that diet was different in

all seasons for which data were collected (Table 5.7). The exceptions were for comparisons

between winter and spring for the small size class in North Coorong, between summer and

autumn for the medium/large size classes in both areas, and between autumn and winter in the

Estuary.

For the small size class in the Estuary, diets in summer and winter were characterised by bivalve

siphons and *N. australiensis*, respectively, while small crustaceans characterised the diet in spring

(Table 5.8). No samples were collected in autumn. SIMPER analyses indicated that the greatest

seasonal dissimilarity in diet occurred between spring and all other seasons (Appendix 5.3; Figure

5.8A). The dissimilarity in diet between spring and the two other seasons was driven mostly by an

increase in the contribution of gammarids, copepods and mysids in spring.

In the Estuary, the diets of medium/large fish during summer, autumn and winter were

characterised by large invertebrates including polychaetes and bivalve siphons. However,

gammarids characterised the diet in spring (Table 5.8). The greatest dissimilarity in dietary

composition for this size class in the Estuary occurred between summer and spring and between autumn and spring (Appendix 5.3; Figure 5.8B). Differences were due mostly to the consumption of larger volumes of gammarids and low volumes of *N. australiensis* in spring (Appendix 5.3; Figure 5.8B).

Table 5.7 PERMANOVA pair-wise comparisons between dietary compositions for the small and medium/large size classes of *R. tapirina* sampled in summer, autumn, winter and spring. No samples in the small size class were available in autumn. Significant *P* values are highlighted in bold (α = 0.05).

Size class	Seasonal pairwise comparison	Estuary		North Coorong	
		t	P	t	P
small	summer, winter	2.06	**0.046**	2.23	**0.033**
	summer, spring	4.83	**<0.001**	2.85	**<0.001**
	winter, spring	2.91	**<0.001**	1.56	0.451
medium/large	summer, autumn	1.48	0.061	1.17	0.053
	summer, winter	2.65	**<0.001**	2	**<0.001**
	summer, spring	6.65	**<0.001**	4.2	**<0.001**
	autumn, winter	1.07	0.063	3.01	**<0.001**
	autumn, spring	5.79	**<0.001**	6.68	**<0.001**
	winter, spring	4.86	**<0.001**	3.93	**<0.001**

Table 5.8 Indicator species analysis of the seasonal variation in dietary composition for the small and medium/large size classes of *R. tapirina* in the Estuary and North Coorong. No prey items were found to be significant indicators for the small size class in North Coorong. Only significant indicator species are shown (α = 0.05).

Study area	Size class	Prey taxa	Season	Indicator value	P
Estuary	small	bivalve siphons	summer	49.8	0.009
		Nephtys australiensis	winter	55.2	0.001
		Mysidacea	spring	55.3	0.003
		Copepoda	spring	51.4	0.009
	medium/large	*Nephtys australiensis*	summer	31.7	<0.001
		bivalve siphons	autumn	12.1	0.026
		Phyllodoce novaehollandiae	winter	10.3	0.027
		Amphipoda: Gammaridea	spring	32.9	<0.001
		unidentified material	spring	19	0.018
North Coorong	medium/large	*Nephtys australiensis*	autumn	36.8	<0.001
		Paragrapsus gaimardii	autumn	19.2	0.001
		Biffarius sp.	autumn	15.8	0.003
		unidentified polychaete	autumn	14.3	0.036
		bivalve siphons	winter	25.2	0.004
		Amphipoda: Gammaridea	spring	39.2	<0.001
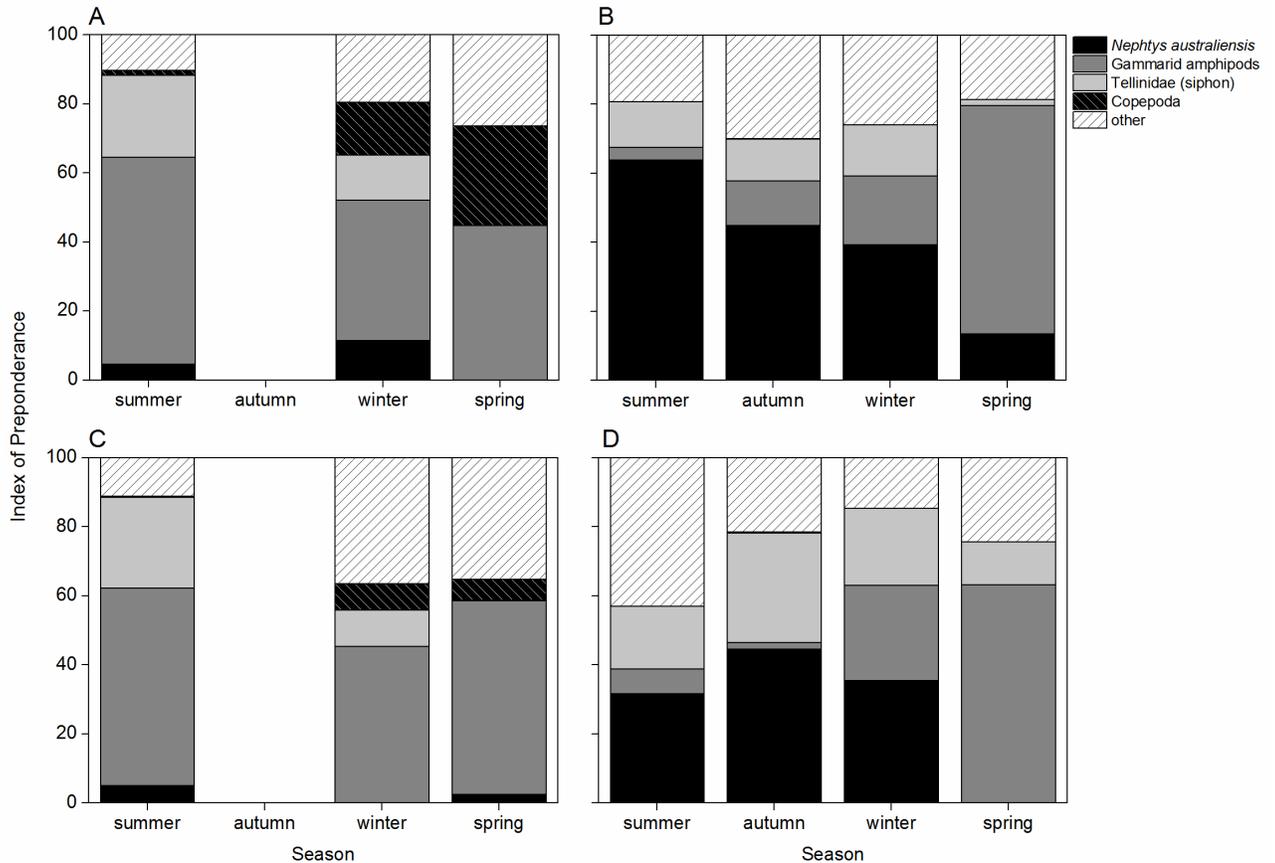
Figure 5.8 Comparisons of the contributions of different categories of prey taxa to the diet of *R. tapirina* between seasons for the (A, C) small and (B, D) size classes for the (A, B) the Estuary and (C, D) North Coorong based on the Index of Preponderance.

For the small size class in North Coorong, no specific categories of prey were significant indicators of the diet in each season. However, SIMPER analyses revealed that the dissimilarity in diet between seasons was highest between summer and spring and was due to a trophic shift from mostly bivalve siphons and gammarids in summer, to a diet of mostly amphipods, copepods and mysids in spring (Appendix 5.4; Figure 5.8C). The dissimilarity between summer and winter was driven mainly by the higher contribution of bivalve siphons in summer and lower contribution of unidentified material in winter.

For medium/large fish in North Coorong, larger invertebrate prey including *N. australiensis*, *P. gaimardii* and bivalve siphons characterised the prey in autumn and winter, while gammarids were significant indicators of the diet in spring (Table 5.8). SIMPER analysis indicated that the greatest dissimilarity in dietary composition occurred between spring and all other seasons

(Appendix 5.4; Figure 5.8D). Each comparison with spring reflected a distinct dietary shift from mostly *N. australiensis* and bivalve siphons, to gammarids in spring.

5.3.4   *Influence of key environmental parameters on seasonal variation in diet*

In the Estuary and North Coorong, DISTLM analysis indicated that dietary composition was influenced by salinity and oxygen concentration (Table 5.9), i.e. the two measured environmental variables that changed most with the commencement of freshwater inflow in spring (Figure 5.3). The influence of water temperature in both areas was not significant (Table 5.9). Salinity had the strongest influence, and together with oxygen concentration accounted for 17.7 % of the variation in dietary composition in both areas. Vector overlays on dbRDA plots for each study area, indicated that salinity contributed most to the differences in diet between spring and all other seasons, particularly for the medium/large size class (Figure 5.9).

Table 5.9 DISTLM sequential results indicating the relative influence of each environmental variable on the variation among groups of *R. tapirina* characterised by different diets for the two study areas. Significant *P* values are highlighted in bold.

	Variable	**Pseudo-F**	**P-value**	**Variation explained (%)**	**Cumulative variation explained (%)**
Estuary	Salinity	47.208	**<0.001**	16.85	16.85
	Oxygen con.	2.415	**0.028**	0.85	17.7
	Water temperature	2.122	0.058	0.75	18.45
North Coorong	Salinity	25.17	**<0.001**	13.52	13.52
	Oxygen con.	8.079	**<0.001**	4.16	17.68
	Water temperature	0.85	0.497	0.48	18.16
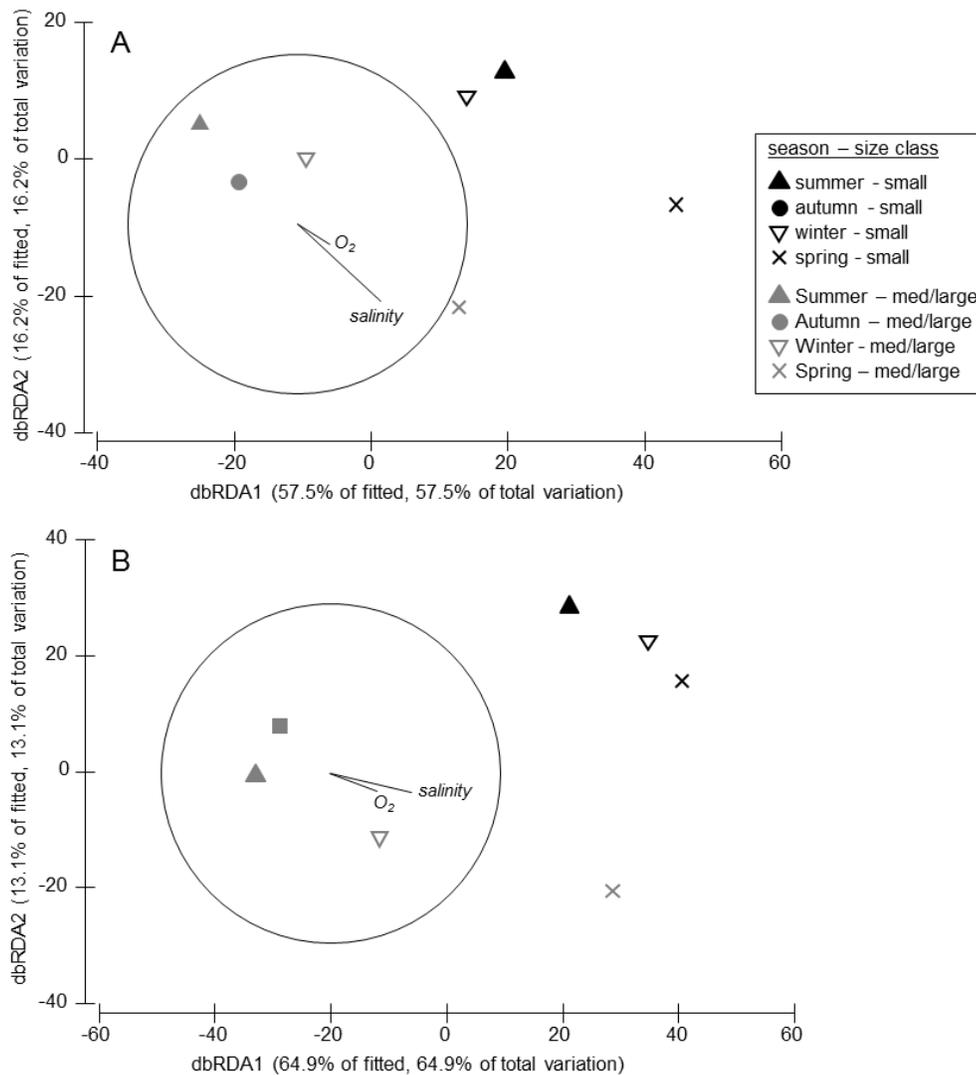
Figure 5.9 Plots of distance-based redundancy analyses (dbRDA) of dietary composition (as mean percentage volume per group) by season for the small (black symbols) and medium/large (grey symbols) for (A) the Estuary and (B) North Coorong. Vector overlays for salinity and oxygen concentration (O_2) indicate the relative strength of their correlation (Spearman rank) with groups of samples.

5.3.5   Stable isotope analysis

Trophic positions of fish

There was considerable variation in the estimates of mean trophic position for *R. tapirina* which ranged between 2.91 (±0.3) and 3.92 (±0.6) in the Estuary (Figure 5.10), and between 3.02 (±0.2) and 3.74 (±0.5) in North Coorong (Figure 5.11). In both areas, the small size class occupied a lower trophic position than the medium and large size classes, however there was often overlap between the trophic positions of medium and large fish.
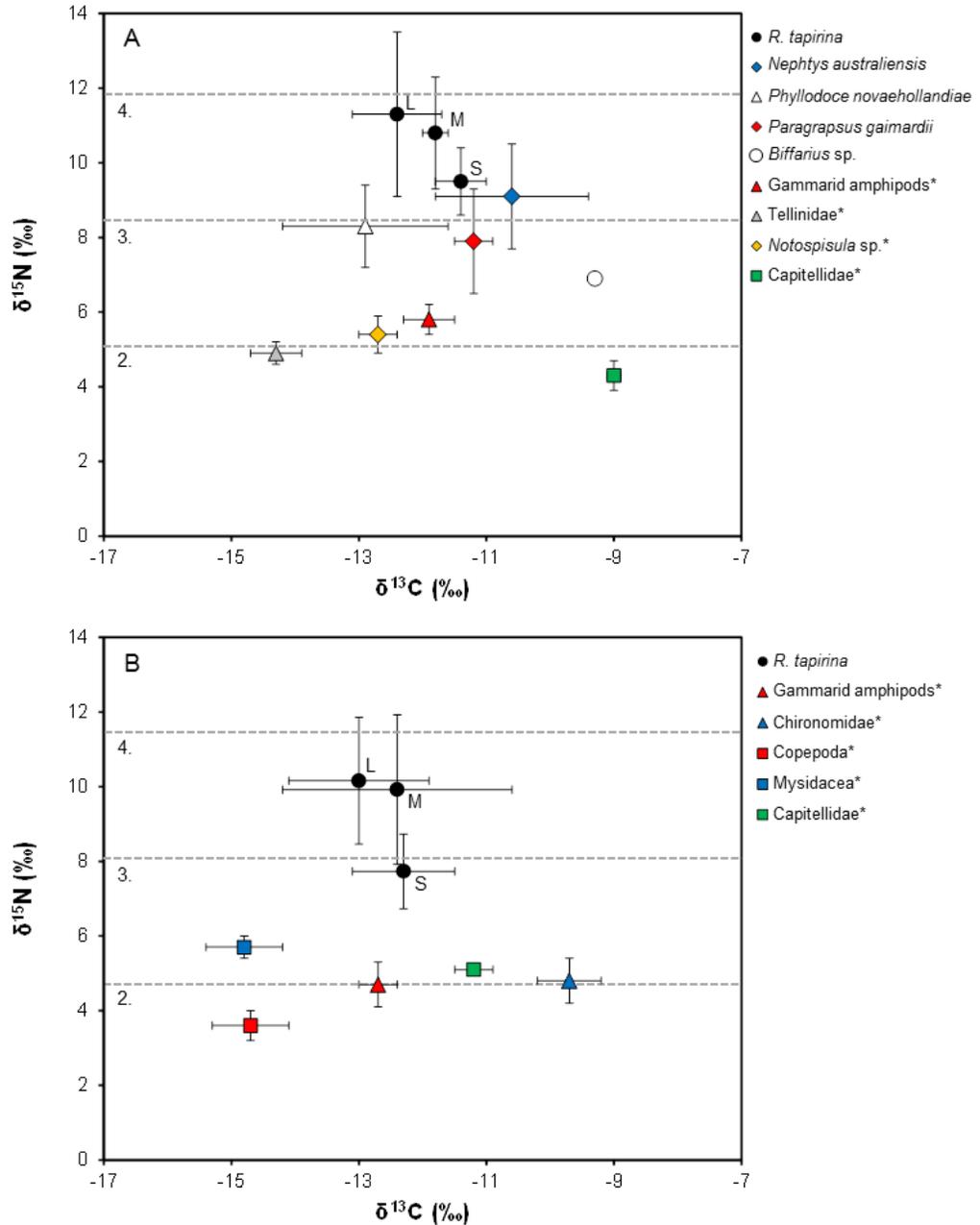
Figure 5.10 Stable nitrogen (δ^{15}N) and carbon (δ^{13}C) isotope signatures of the (S) small, (M) medium and (L) large size classes of *R. tapirina* and categories of potential prey items collected in (A) summer and (B) spring from the Estuary. Trophic levels are presented for δ^{15}N in increments of 3.4‰. Error bars represent standard deviations.
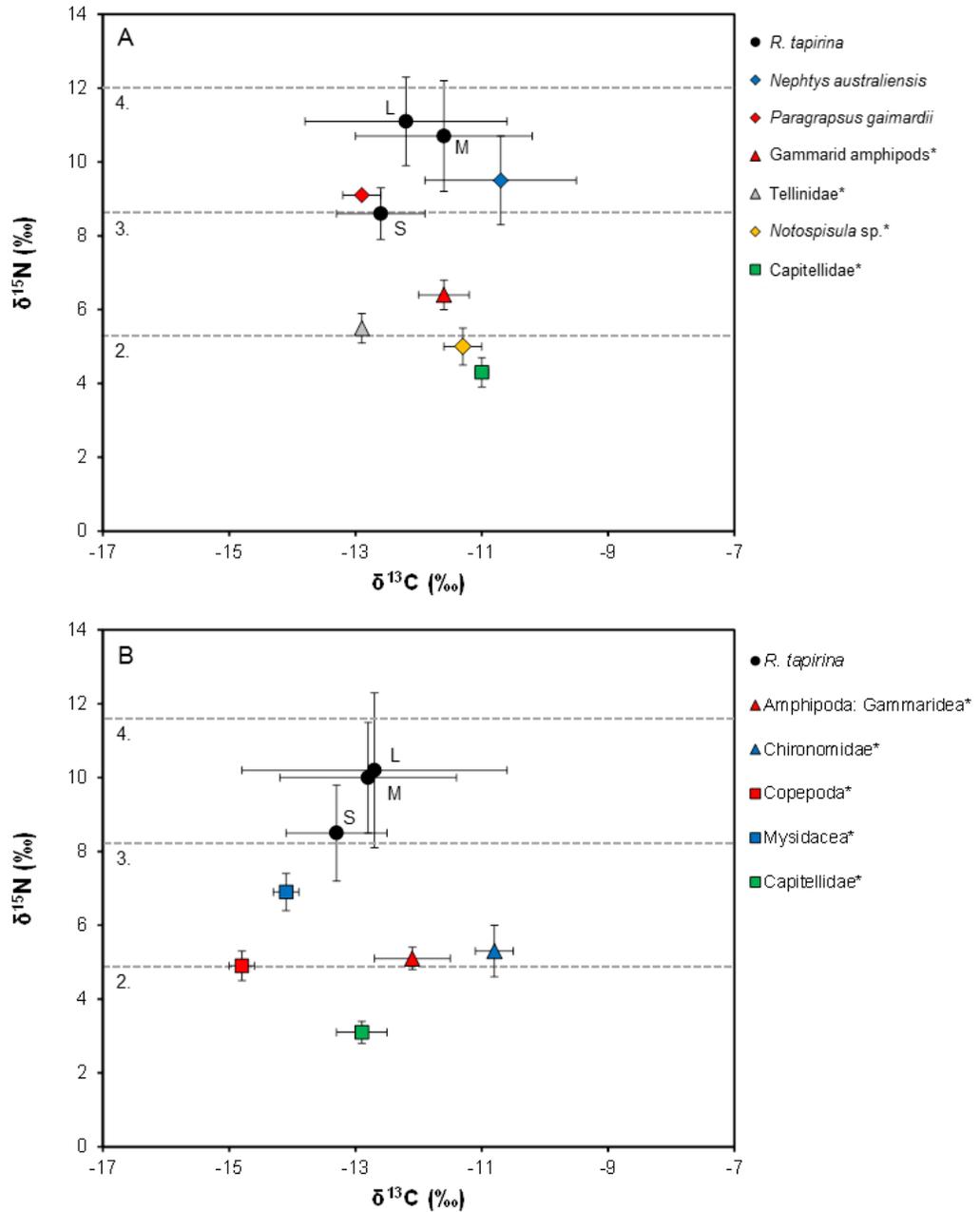
Figure 5.11 Stable nitrogen (δ^{15}N) and carbon (δ^{13}C) isotope signatures of the (S) small, (M) medium and (L) large size classes of *R. tapirina* and categories of potential prey items collected in (A) summer and (B) spring from North Coorong. Trophic levels are presented for δ^{15}N in increments of 3.4‰. Error bars represent standard deviations.

There was a significant effect of fish size on trophic position, but no main effect of area, season or of the interaction between area, size class and season (Table 5.10). Pairwise comparisons indicated that size-related differences in trophic position occurred between the small and medium size classes (t = 8.32, P < 0.001) and between the small and large size classes (t = 7.53, P < 0.001), however there was no difference between the medium and large size class (t = 0.56, P = 0.583).

Table 5.10 PERMANOVA results for comparison of trophic position of *R. tapirina* between area, size class and season in the Coorong estuary. Significant *P* values are highlighted in bold (α = 0.05).

Factor	d.f.	MS	Pseudo-*F*	P
Area	1	61.2	2.99	0.087
Size class	2	838.4	40.92	**<0.001**
Season	1	58.7	102.42	0.069
Area x Size class	2	2.1	0.1	0.919
Size Class x Season	2	70.2	3.43	0.07
Area x Season	1	27.5	1.34	0.264
Area x Size class x Season	2	8.3	0.41	0.676
Residuals	48	20.5		

Diet composition based on SIA

Stable nitrogen (δ^{15}N) and carbon (δ^{13}C) isotope signatures for *R. tapirina* and numerous invertebrate food sources in the Estuary and North Coorong were examined to assess their trophic relationships. Potential contributions of the different food sources to the diet were estimated based on standard fractionation assumptions of < 1‰ for δ^{13}C and ~3.4‰ for δ^{15}N (Post 2002; McCutchan et al. 2003).

For the Estuary during summer, gammarids and the shore crab *P. gaimardii* were identified as the most important prey items in the diets of all three size classes (Figure 5.10A). For the two larger size classes, *N. australiensis, Phyllodoce novaehollandiae* the bivalve *Notospisula* sp. were identified as important items, whilst the contribution of capitellids, *Biffarius* sp. and tellinid bivalves was low. The diet of the small size class was likely to have also included *P. novaehollandiae,* although their contribution was likely lower (Figure 5.10A).

During spring in the Estuary, the assimilated diets of all three size classes shifted to include more gammarids and capitellids (Figure 5.10B). For the large and medium size class, mysids were an important dietary item, while the contribution of all other prey items was low. The isotopic signatures of all other prey items, including copepods, did not appear to have contributed to the diet of any size class (Figure 5.10B).

For North Coorong during summer, tellinid bivalves and gammarids were important dietary items for all three size classes of *R. tapirina* (Figure 5.11A). For the medium and large size classes, *P. gaimardii* and *N. australiensis* were important contributors to the diet, however this is unlikely to have been the case for capitellids and *Notospisula* sp.. Capitellids and *Notospisula* sp. were also assimilated by the small size class, but *P. gaimardii* and *N. australiensis* were unlikely to have been involved in their diet (Figure 5.11A). During spring, the diets of all three size classes included gammarids, copepods and mysids (Figure 5.11B). Chironomid larvae were also important prey to the two larger size classes, though it is unlikely that they were a key category in the diet of small fish (Figure 5.11B).

5.4   Discussion

Initially, this study aimed to use a combination of gut contents analysis and SIA to describe dietary composition and feeding habits for *R. tapirina* in the Coorong estuary. However, this approach was modified due to the relatively low number of potential food sources available for SIA. As such, gut contents analysis was the primary method used to examine the diet and SIA was used as a complementary method to identify variation in trophic level and determine the likely assimilatory contribution of different prey categories to the diet. This did not compromise the integrity and usefulness of the study, as the main limitations of gut contents analysis in determining fish diet (Cocheret de la Moriniere et al. 2003; Fanelli et al. 2011) were overcome. First, samples of *R. tapirina* were collected over a long period to account for temporal variation in diet, and second, the majority of the prey items in the guts of fish were identifiable to at least a broad taxonomic category.

Both gut content data and SIA indicated that *R. tapirina*, like other temperate flatfish, is a carnivorous, generalist feeder that exploited food resources from a wide array of taxonomic groups (Link et al. 2005). The main components of the diet were polychaetes, gammarid amphipods and bivalve siphons. Whilst, on average, these prey items accounted for > 90 % of the diet, other small crustaceans, small annelids and insect larvae were also consumed but in

relatively low volumes. Polychaetes and amphipods are important contributors to the diets of most flatfish species (e.g. Pearcy and Hancock 1978; Livingston 1987; Carlson et al. 1997; Link et al. 2002). However, predation of bivalve siphons is less common and is a behaviour generally associated with juveniles of specialist feeders (Hill et al. 2000; de Goeij et al. 2001).

Despite the relatively high contribution of only a few prey categories to the diet of *R. tapirina*, there were considerable differences in dietary composition among size classes. Gut content analysis identified a distinct shift from the consumption of mainly gammarids, copepods and bivalve siphons by small fish, to a diet that also included polychaetes and crabs for larger individuals. Estimates of $\delta^{15}N$, as determined from SIA, were considered to verify these differences, as $\delta^{15}N$ provides a reliable indicator of trophic position for fish (Post 2002; McCutchan et al. 2003). The increase in $\delta^{15}N$ with fish size for *R. tapirina* was consistent with the results of gut contents analysis and indicated a gradual increase in the trophic position as fish grew. This corroborated the ontogenetic shift of *R. tapirina,* from a diet that consisted of mostly primary consumers with low $\delta^{15}N$, i.e. amphipods and bivalves, towards a diet that also involved prey which were positioned higher in the food chain such as polychaetes.

All flatfishes consume polychaetes and small crustaceans at some point in their life histories (Link et al. 2005). However, numerous species feed on these prey items at smaller sizes but grow out of this feeding mode to consume larger prey such as fish, squid and echinoderms (Livingston 1987; Yamada et al. 1998; Orlov and Moukhametov 2004). This pattern represents the classic ontogenetic trophic shift, but is generally more common for larger-bodied flatfish species such as *Hippoglossus hippoglossus* (Link et al. 2002) whose feeding apparatus as juveniles is vastly different in size and structure to that of adults. In comparison, *R. tapirina* is a relatively small/medium-sized flatfish species, for whom the difference in size of the feeding apparatus between juveniles and adults is less pronounced. As a consequence, differences in the type and size of prey consumed between size classes, whilst still increasing with fish size, were much more subtle compared to many other species. As such, polychaetes and small crustaceans remained important dietary constituents beyond the juvenile stage.

For species that exhibit changes in habitat preference with ontogeny, fine-scale variation in the availability of food resources may also contribute to size-related differences in diet (Wootton 1990; Elliott et al. 2002). In the Coorong estuary, juvenile *R. tapirina* are most abundant in sandy shallow habitats, such as on tidal flats and banks (Ye et al. 2011), while larger individuals occupy

deeper habitats such as channels and holes during the day, before moving up to shallower habitats during the night (Chapter 6), presumably in search of prey (Burrows et al. 1994; Vinagre et al. 2006). Whilst variation in the abundance of larger invertebrates such as polychaetes, i.e. the main contributor to the diet for larger *R. tapirina*, between shallow flats and deeper habitats in the Estuary and North Coorong is not known, the overlap in habitats of small and large fish suggests that each size class has access to similar food resources, at least during hours of darkness. As such, size-related changes in diet for *R. tapirina* are unlikely related to habitat differences, but rather reflect the subtle ontogenetic changes in the size and functional morphology of the feeding apparatus.

Larger-scale spatial differences in diet for estuarine fish can also reflect differences in the availability of prey (Bottom and Jones 1990; Edgar and Shaw 1995; Griffiths 1997). Despite the contrasting salinity regimes of the two study areas, differences in diet for small and large *R. tapirina* between the Estuary and North Coorong were marginal. In each area, the three major prey categories for *R. tapirina,* i.e. polychaetes, gammarids and bivalve siphons, were the main contributors to the diet, though their relative volumetric contribution to the diet often varied. In addition, the respective trophic positions of small, medium and large *R. tapirina* were similar in the Estuary and North Coorong, suggesting that fish consumed prey of similar trophic positions in each area. Similarities in diet for *R. tapirina* between the areas suggest that prey items were available across this spatial scale and/or that fish moved regularly between these two areas. The co-occurrence of small crustaceans and polychaetes in both areas during the study period, albeit in relatively low abundances (Dittmann et al. 2011b), combined with the highly motile behaviour of some *R. tapirina* (described in Chapter 6) suggests that both of these explanations are possible.

Seasonal changes in diet were also detected for *R. tapirina.* In estuaries, such changes can relate to the effects of temperature and salinity on feeding rates (Lankford and Targett 1994) and/or temporal fluctuations in the availability of prey (Sarre et al. 2000). For small and large *R. tapirina*, differences in diet between summer, autumn and winter were marginal, as the contribution of the main dietary categories were relatively consistent among seasons for each size class. As water temperatures in the Coorong estuary ranged from $< 10\ ^{o}C$ to $28\ ^{o}C$ during this period, it was clear that temperature did not have a major influence on the feeding habits of the fish. Nonetheless, the species exhibited a distinct trophic shift in spring, from a diet consisting of mainly bivalves, gammarids and polychaetes through summer, autumn and winter, to a diet of almost exclusively gammarid amphipods and copepods. The timing of this shift coincided with

the commencement of large, continuous freshwater discharges from the lower Murray River into the Coorong estuary after a prolonged period of drought. These flows immediately reduced salinity from near-marine and hypersaline in Estuary and North Coorong, respectively, to mostly fresh in both areas. Oxygen levels also declined abruptly, presumably due to the high organic load of the turbid flood waters that entered the system (Johnston et al. 2003).

The direct effects of these flow-related environmental changes on the feeding habits of *R. tapirina* are not clear, but most likely relate to changes in the distribution and abundance of invertebrate-prey (Kimmerer 2002). In a review of the trophic ecology of polychaete-feeding flatfish species, Link et al. (2002) concluded that amphipods and other small crustaceans augment the polychaete portion of the diet during times or at locations where polychaetes are less abundant or not available. Most large polychaete species that occur in the Coorong estuary show a preference for brackish and/or marine-like conditions (Dittmann et al. 2012). An increase in abundances of amphipods after high flows was noted in late 2010 as well as after earlier water releases (Geddes 1987; Dittmann et al. 2012). Thus, the abrupt freshening of some parts of the system during periods of high flows may have displaced polychaetes, forcing adult *R. tapirina* to either modify their diet to include food items that are more accessible or move to areas where their preferred prey are more abundant. Unfortunately the temporal constraints of this study precluded assessing changes in distribution of *R. tapirina* in response to changes in prey availability.

In summary, this study demonstrated that *R. tapirina* is a carnivorous, generalist feeder, whose diet was dominated by polychaetes, amphipods and bivalve siphons, but included a broad and diverse range of macrobenthic invertebrate taxa. This species exhibited a distinct ontogenetic trophic shift that likely related to changes in feeding morphology. Spatial differences in diet were marginal across the salinity-mediated, contracted distribution of *R. tapirina* in the Coorong estuary during the study, suggesting that the availability of prey categories was similar across this spatial scale and/or that fish moved regularly between the two study areas. Seasonal variation in water temperature did not appear to directly influence the feeding habits of *R. tapirina.* However, there were distinct differences in diet before and after the commencement of drought-breaking freshwater inflows in spring, which most likely related to changes in prey availability in response to the abrupt changes in salinity and oxygen. The environmentally-mediated variation in the diets of juvenile and adult *R. tapirina* imply that this species is able to feed opportunistically and adjust its diet to deal with abrupt environmental change, a characteristic that is of particular value to fish that live in estuaries where the availability of different prey types can be highly variable.

6 Movement, residency and habitat use of greenback flounder (*Rhombosolea tapirina*) in a large temperate estuary during a period of high freshwater flow

6.1 Introduction

Understanding the spatial behaviour and habitat use of estuarine fish is critical for the effective management of their populations (Beck et al. 2001). Knowing when fish move and where they occur can inform about demographic processes, resource use and their ability to withstand and respond to environmental change (Meyer et al. 2000; Gillanders et al. 2011; Walsh et al. 2013). The scale of fish movement can range from highly localised movements, often associated with foraging and predator avoidance, to long-distance seasonal or life-cycle related migrations from estuaries to the outer continental shelf (Elliott et al. 2007; Becker et al. 2011; Furey et al. 2013). Such plasticity in behaviour not only reflects the diverse life history strategies and physiological capabilities of fish, but also the dynamic nature of the environments they occupy. Despite the availability of numerous techniques for studying fish behaviour, recent advances in biotelemetry have greatly improved the ability to monitor fish movement across a range of aquatic systems (Lucas and Baras 2000; Cooke et al. 2004; Heupel et al. 2006; Able et al. 2005).

Acoustic telemetry, which dates back to the 1950s, has become the most common method for studying the behaviour of fish in their natural environment, as it provides real-time analysis of behaviour across a range of temporal and spatial scales (Trefethen 1956; Heupel et al. 2006). This methodology involves the complementary use of electronic tags implanted in fish and static underwater acoustic receivers. Each tag transmits an acoustic pulse encoded with a unique ID code, which is detected and recorded by receivers to provide positional fixes for fish through time (Humston et al. 2005). The spatial and temporal distribution of the positional fixes are then interpreted in terms of fish movement within areas of interest, as well as providing information on behaviour and habitat use relative to environmental information, such as depth and water temperature data.

Flatfish are common in many temperate estuaries and have been known to dominate the demersal fish fauna in these highly productive ecosystems (Munroe 2005). The use of estuaries by juvenile

flatfish is well documented as these environments provide food-rich, sheltered habitats which enhance early growth and survivorship (Ramos et al. 2010; Mariani et al. 2011; Primo et al. 2013). However, numerous species utilise estuaries beyond the juvenile stage. Some species occupy mostly offshore habitats and migrate periodically to estuaries to spawn or forage (Hanson and Courtenay 1996; Kraus and Musick 2001), while others complete their entire life cycle within estuaries and are reliant on specific sets of environmental conditions to facilitate reproduction, recruitment and growth (Armstrong 1997). Such differences highlight the need to understand the contribution of movement and residency patterns of individual species to their population dynamics.

The greenback flounder, *Rhombosolea tapirina* is a medium-sized, demersal flatfish of the family Pleuronectidae (Gomon et al. 2008). This species is known for its strong association with estuaries and nearshore coastal environments throughout southern Australia and New Zealand (van den Enden et al. 2000; Gomon et al. 2008), where it supports valuable commercial and recreational fisheries (Kailola et al. 1993; Froese and Pauly 2013). In South Australia, almost all catches are taken by the multi-species Lakes and Coorong Fishery (LCF) which operates in the Coorong estuary and adjacent areas (Ferguson 2007). Fishers in the LCF harvest *R. tapirina* using mainly large-mesh gill nets, whilst recreational fishers also target this species in shallow water at night using spot-lights and spears (Kailola et al. 1993). Historical data for the LCF indicate that population abundance of *R. tapirina* is highly variable among years (Ferguson 2007). Whilst freshwater discharge into the Coorong estuary has a significant influence on the population abundance (Chapter 2), knowledge of key demographic processes that drive changes in population size is lacking.

In South Australia, *R. tapirina* is regarded as an estuarine resident, i.e. it completes its entire life cycle within estuaries (Hall 1984). However, the high inter-annual variation in abundance (Chapter 2) may relate to movement of fish between the estuary and marine environment in response to variation in the availability and quality of habitat in the estuary. Whilst this hypothesis is supported by the extreme variation in commercial catches of *R. tapirina* in the system among years, e.g. from 0.1 t in 2010/11 to 31 t in 2011/12, little is known about the migratory capabilities and movement patterns of the species. This study used acoustic telemetry to examine the movement patterns of *R. tapirina* within the Coorong estuary. The specific aims for *R. tapirina* were to: (1) describe the movement patterns of individuals; (2) examine spatial and temporal

patterns of residency and how they are influenced by flow-mediated changes in salinity; and (3) to describe the fine-scale patterns in habitat use with respect to water depth and time of day.

6.2   Materials and methods

6.2.1   Study area

This study was carried out in the Coorong estuary (35° 34'S; 138° 55'E), in South Australia (Figure 6.1). Situated at the terminus of Australia's largest river system, the Murray-Darling, the Coorong estuary comprises a long and narrow lagoon that extends south-east from the Murray Mouth, approximately 110 km. The original Murray River estuary covered 660 km^2 and included the brackish Lakes Alexandrina and Albert and the Coorong lagoon. In the early1940s, a series of barrages were constructed between the Lakes and the Coorong lagoon, which reduced the estuary to 11 % of its original size (Leblanc et al. 2012). Barrage construction, river regulation and extractive water use from the Murray-Darling Basin have resulted in a reduction in mean annual freshwater discharge to the Coorong estuary from ~12,233 GL to ~4,723 GL and an increase in frequency of years of no freshwater inflow (CSIRO 2008).

In recent decades, the timing, magnitude and frequency of freshwater inflows to the Coorong estuary have been highly variable (Figure 6.2). The recent Millennium Drought from 2002 to 2010 (Leblanc et al. 2012) resulted in several years of minimal inflow and a 3-year period from 2007 to mid-2010 when no inflows occurred. The drought contributed to a longitudinal salinity gradient within the estuary that extended south-east from the mouth (Webster 2010), with salinity ranging from marine near the Murray Mouth, to hypersaline in the southern parts of the Coorong (Kingsford et al. 2011). In September 2010, a drought-breaking flood event in the Murray-Darling Basin resulted in the resumption of freshwater inflow to the Coorong. The present study was conducted from May 2011 to January 2012 during a period of high freshwater inflow. During that period, inflow peaked at ~2,200 GL month^{-1} in early 2011 and remained predominantly >300 GL month^{-1} until the end of that year.
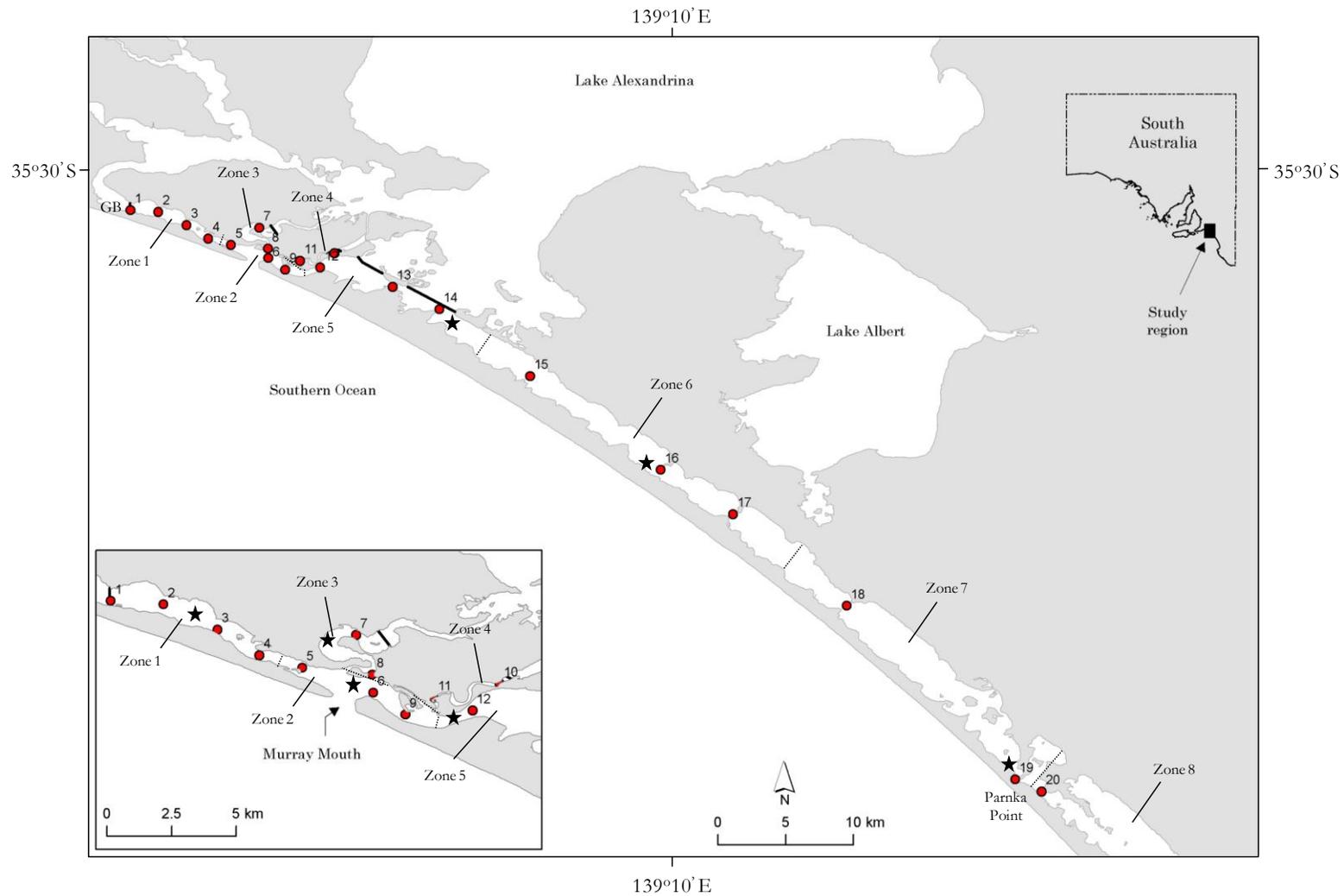
Figure 6.1 Map of the study area showing the locations of the twenty receiver stations (red dots); the eight spatial zones divided by the broken black lines; and the locations of the continuous water monitoring stations (black stars) located within each spatial zone. Inset shows the location of Murray Mouth in relation to the receivers located in the Estuary. The locations of the five barrages are indicated by thick black lines. GB – Goolwa Barrage.
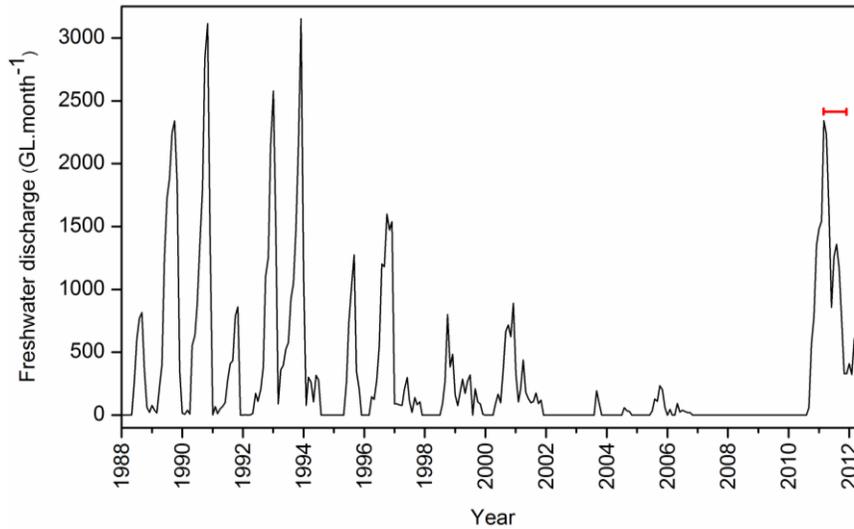
Figure 6.2 Estimates of total monthly freshwater discharge to the Coorong estuary from January 1988 to March 2012 (data source: Murray-Darling Basin Authority 2013). Capped red line indicates the timing of the study period.

For this study, the Coorong estuary was divided into eight 'spatial zones' based upon geographic features and the positioning of acoustic receivers (Figure 6.1). These zones were used to investigate patterns of movement and residency of the fish.

6.2.2   Salinity data

Daily mean electrical conductivity data for the period from May to December 2011 were obtained from continuous water monitoring stations located within each of the eight spatial zones by the Department of Environment, Water and Natural Resources (Government of South Australia 2013). Where two monitoring stations were located in one zone, the mean salinity was calculated for each day. Owing to technical problems, no data were available for Zone 4. Daily mean electrical conductivity was used as a proxy for salinity and is referred to as salinity hereafter (freshwater = 0 µS/cm; seawater ~50,000 µS/cm).

6.2.3   Application of acoustic telemetry

Capture and tagging of fish

The movement patterns, residency and habitat use of adult *R. tapirina* were monitored in the Coorong estuary using passive acoustic telemetry from May to December 2011. Each of a number of fish was tagged with an acoustic tag. These fish were captured using a fine-mesh beach seine net at numerous locations throughout the Coorong estuary. Once captured, each fish was immediately placed into a 100-L aerated-holding tank and monitored. Only undamaged, free-

swimming fish were considered for surgery to insert the acoustic tag. In total, 20 adult fish that

ranged from 221 to 313 mm in total length (TL) were tagged (Table 6.1).

Table 6.1 Summary of the biological characteristics and release details of *R. tapirina* that were tagged and
monitored in the Coorong estuary. All tagged fish were sexually mature females. See Figure 6.1 for
locations of receivers.

Fish ID	Total length (TL)	Total weight (g)	Tagging date	Release location (receiver no.)	Total detections	No. of days monitored
3740	243	172	17/05/11	16	1126	213
3741	234	188	04/05/11	16	171	16
3742	231	178	02/06/11	16	230	9
3743	221	154	02/06/11	16	1067	45
3744	246	209	20/07/11	16	441	6
3745	244	175	05/05/11	18	234	119
3746	224	138	18/05/11	16	190	19
3747	223	135	19/05/11	16	1666	116
3748	284	264	13/07/11	17	1996	87
3749	231	197	20/07/11	16	856	80
3750	294	269	05/05/11	17	329	30
3751	270	201	18/05/11	16	1593	128
3752	225	168	13/07/11	16	1137	162
3753	236	219	13/07/11	16	942	7
3754	313	367	23/06/11	3	1029	13
3755	310	292	04/05/11	5	250	1
3756	231	168	18/05/11	16	71	6
3757	233	171	20/07/11	16	2297	76
3758	245	270	13/07/11	16	197	145
3759	236	175	20/07/11	16	820	38

To prepare for surgery, each fish was individually anaesthetized using a 0.05 ml L^{-1} solution of

AQUI-S$^{©}$ (AQUI-S, New Zealand Ltd, Lower Hutt, New Zealand) in a 25 L aerated dosing tank.

Once the fish exhibited loss of reflex, it was weighed to the nearest gram, measured for total TL

to the nearest mm, and a continuous flow of 0.025 ml L^{-1} solution of AQUI-S$^{©}$ was irrigated over

the gills. A sterilized, individually coded VEMCO$^{©}$ V9P-1L acoustic transmitter with coded

random signal delay of 60 – 180 seconds was inserted into the peritoneal cavity via a small

incision on the dorsal side below the pectoral fin (Figure 6.3). Each transmitter had a projected

battery life of 194 days, was 9 mm in diameter, 42.5 mm in length and weighed approximately

2.8 g in water (on average 1.5 % of the fish's body mass (min 0.76 %, max 2.07 %)). The incision

was then closed using a single cruciate suture and the wound was sealed with a tissue adhesive

(3M Vetbond[©] 3M, Minnesota, USA). After surgery, a dose of the antibiotic Baytril[©] (0.1 mL kg)
was injected into the dorsal muscle tissue to minimise possible infection. The fish was returned to
the aerated holding tank and monitored until it had resumed normal swimming orientation and
regained reflex movements. All fish recovered from the surgical procedure and were released at
their capture locations.

Figure 6.3 Photograph of a female *R. tapirina* (270 mm TL) prior to surgery to insert the acoustic tag into
its peritoneal cavity. The location of the incision (solid black line) through which the transmitter was
inserted is also shown. Rectangular shaded area indicates the likely position of the transmitter within the
peritoneal cavity.

Receiver array

A fixed array of submerged acoustic receivers (VEMCO[©] VR2W) was used to detect and record
information from ultrasonic (69 kHz) signals emitted by the acoustic transmitters in tagged fish in
real time. The spatial coverage of the receiver array and the position of individual receivers had
been established earlier for a study on black bream and congolli. A total of twenty receivers had
been placed strategically throughout the Coorong estuary, from downstream of the Goolwa
Barrage to Parnka Point, covering approximately 75 km (Figure 6.1). The spatial resolution of this
array was often limited, due to distances of up to 12 km between some receivers. The number of
receivers positioned within each of the eight spatial zones ranged from one to four. Range testing
of receivers indicated 100 % detection efficiency to 350 m, declining to approximately 60 % at
400 m. As such, receiver locations had been selected where channel widths did not exceed 600 m,

to ensure tagged fish could not move past a receiver and avoid detection. Where this was not possible, a second receiver was deployed to increase the detection range at a specific location. Individual receivers were attached underwater to concrete moorings and orientated vertically in the water column to sit approximately 1 m above the substrate. Three types of data were recorded when a fish moved within the detection range of a receiver: the unique identification code of the transmitter in the fish; the time of the detection; and the depth of the fish at the time of the detection. Receivers were downloaded on two occasions during the study period and once in early 2012.

6.2.4   Data analysis

Data for the 24-hour period following the release of tagged fish were not considered for analysis because of possible atypical behaviour following surgery (Hartill et al. 2003).

Movement patterns

The movement patterns of individual *R. tapirina* within the Coorong estuary were examined by plotting the time and location of detections by individual receivers. These plots provided a qualitative representation of the temporal sequence of fish movements between receivers throughout the study region. Due to inconsistent spacing between receivers in the system, all subsequent analyses of fish movement and residency were carried out with respect to the eight spatial zones (Figure 6.1).

Variation in the movement activity of *R. tapirina* in the Coorong estuary was assessed by determining the number of different spatial zones visited by each fish in each month. A single-factor univariate PERMANOVA (permutational analysis of variance) was used to test for differences ($\alpha=0.05$) in the mean number of zones visited between months. Where a significant difference was detected, pairwise analyses were used to determine which months were statistically different (Clarke and Gorley 2006). Prior to analysis, visit data were square root transformed, and Bray-Curtis similarities were calculated between pairs of zones to produce a similarity matrix.

Residency

Spatial and temporal patterns in area use by individual *R. tapirina* were described using an index of residency. Residency was estimated based on the proportion of time each tagged fish spent within each of the eight spatial zones during each month throughout the study period. Due to the

large distances between consecutive receivers in some zones, fish were not continuously detected by receivers when positioned between two receivers and thus outside of their detection ranges. A tagged fish was assumed to be present within a particular spatial zone when two (or more) consecutive detections were recorded on receivers within that zone within one hour. Absence times of more than one hour were assigned in equal proportions to the zone frequented before and after the absence period. If a fish returned to the same zone, the entire absence time was assigned to that zone. If a fish went undetected for an extended period of time (days) it was assumed to be either stationary between two receivers, or at sea. Recaptures of tagged fish during the study indicated that some fish remained within a zone for at least 2 months. As such, no rule restrictions were placed on fish that went undetected for extended periods of time, rather it was assumed to be alive but relatively stationary between receivers. A trip from the estuary to the adjacent marine environment was confirmed if detections were recorded on receiver 5 or receiver 6, i.e. either side of the Murray Mouth, before and/or after an undetected period exceeding 24 hours. If fish were last detected at the Murray Mouth and not detected again, they were assumed to have emigrated from the system, and a standard residence time at sea of two weeks was assigned.

Temporal variability in spatial residency patterns of *R. tapirina* was assessed by investigating the proportion of time spent by each individual within each spatial zone in each month. A two-factor univariate PERMANOVA was used to test for differences (α=0.05) in the proportion of time spent by fish (replicates) between zones (fixed factor) and between months (random factor). Where a significant difference was detected, pairwise analyses were used to determine which months were statistically different (Clarke and Gorley 2006). Prior to analysis, proportion data were arcsine transformed, and Euclidean distances were calculated between pairs of zones to produce a distance matrix. All analyses were performed using the Plymouth Routines in Multivariate Ecological Research (PRIMER v.6 and PERMANOVA+) Software.

Residency patterns in relation to salinity

To examine the influence of salinity on the residency of *R. tapirina*, a daily estimate of salinity exposure was allotted to every day spent within the study area for each fish. These daily estimates were obtained from the nearest continuous monitoring station to which the fish was detected. A frequency histogram was calculated based on the proportion of time tagged fish spent in a specific salinity range. Estimates of salinity exposure were binned into specific intervals of 10,000 µS/cm (e.g. 0 – 10,000 µS/cm).

Fine-scale patterns in habitat use

Estimates of water depth transmitted from the acoustic tags were used to identify fine-scale patterns in habitat use in the Coorong estuary. To determine the range of water depths in which *R. tapirina* were positioned during the study, all estimates of depth, i.e. from all receivers and all fish, were binned into specific depth intervals of 0.25 m (e.g. 0 - 0.25 m). The frequencies (%) of detections assigned to the different depth intervals were plotted.

The same method was applied to examine diurnal variation in fine-scale habitat-use in the main aggregation area, i.e. in the vicinity of receiver 17. This receiver recorded 31 % of all detections and was located > 3 km from the main tag-and-release location. Depth distributions were prepared from detections recorded during the 'Day', i.e. 06:00 - 17:59 h, and 'Night', i.e. 18:00 – 0559 h, and were compared using the Kolmogorov-Smirnov 2-sample goodness-of-fit test (α=0.05). The mean depth (± S.E.) at which fish were detected was calculated for each hour of the day to demonstrate the magnitude of diurnal variation in habitat use.

6.3   Results

6.3.1   Salinity

Estimates of mean monthly salinity recorded in the Coorong estuary in 2011 ranged from 369 μS/cm to 123 936 μS/cm and differed considerably among spatial zones (Figure 6.4). As a result of the relatively high freshwater inflows to the estuary in each month during the study period (Figure 6.2), mean monthly salinity levels for spatial zones adjacent to the barrages, i.e. Zones 1-5, were relatively low and rarely exceeded 25,000 μS/cm. Beyond these zones, salinity increased with increasing distance from the Murray Mouth. In Zone 6, mean monthly salinity was slightly below or equal to that of seawater in most months, whilst salinity levels in Zones 7 and 8 were almost twice that of seawater, with little variation between months (Figure 6.4).

6.3.2   Summary of detections

All tagged fish survived surgery and were subsequently detected by the receiver array (Figure 6.5). Overall, a total of 16,642 detections were recorded across 15 of the 20 receivers (Figure 6.6). The numbers of detections differed amongst receivers, with 63 % of the total number recorded by Receivers 16 (*n* = 5,315 detections) and 17 (*n* = 5,140) (Figure 6.6). No recordings occurred south of Receiver 18. The numbers of detections recorded for the different tagged fish ranged from 71 to 2297, whilst the time between the first and last detections for individual fish ranged from 28

hours to 212 days (Figure 6.5). Two tagged fish (Fish 3758 and 3751) were captured by commercial fishers, 145 days and 128 days after release, respectively. Both fish were captured approximately 10 km south of their release locations and were not returned to the water. Fish 3744 and 3747 were recaptured by the author approximately 2 km from their release sites, and were returned to the water immediately.
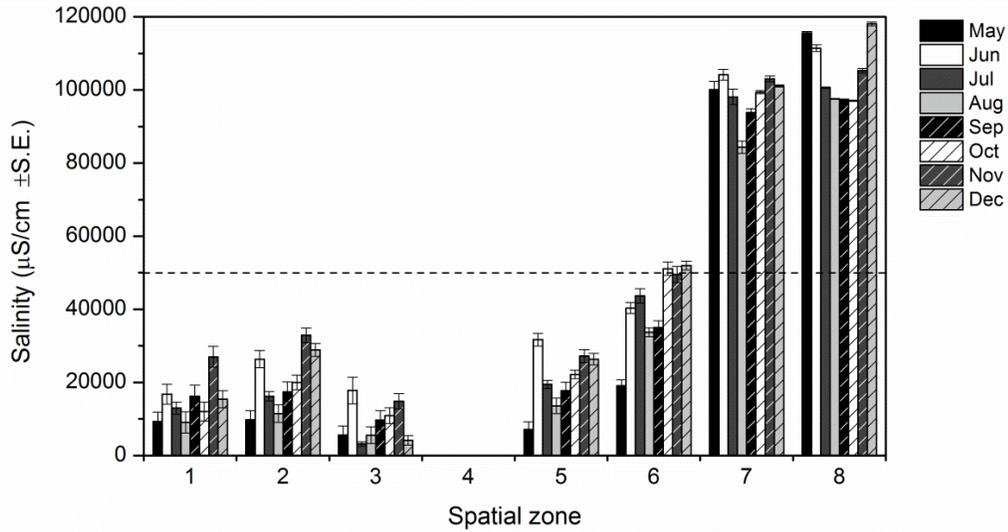
Figure 6.4 Monthly estimates of mean daily salinity (± S.E.) for the eight spatial zones recorded on continuous water monitoring stations in each zone from May to December 2011. Broken grey line indicates the approximate salinity of seawater (data source: Government of South Australia 2013). No salinity data were available from Zone 4.
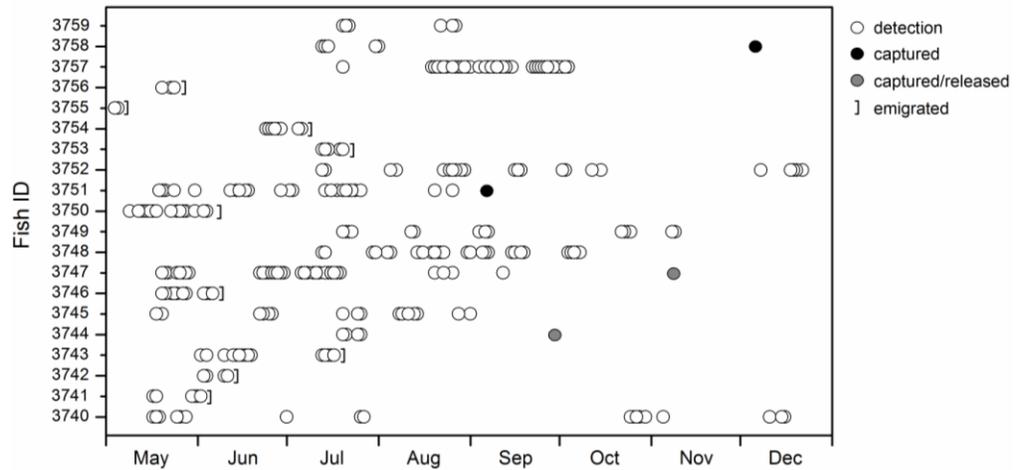
Figure 6.5 Summary of the timing of detections for tagged *R. tapirina* in the Coorong estuary from May to December 2011, including key events that occurred for some fish during the study period (captured = harvested by the fishery and not returned to the water; captured/released = captured by the author and return to the water immediately; emigrated = last detected at the Murray Mouth).
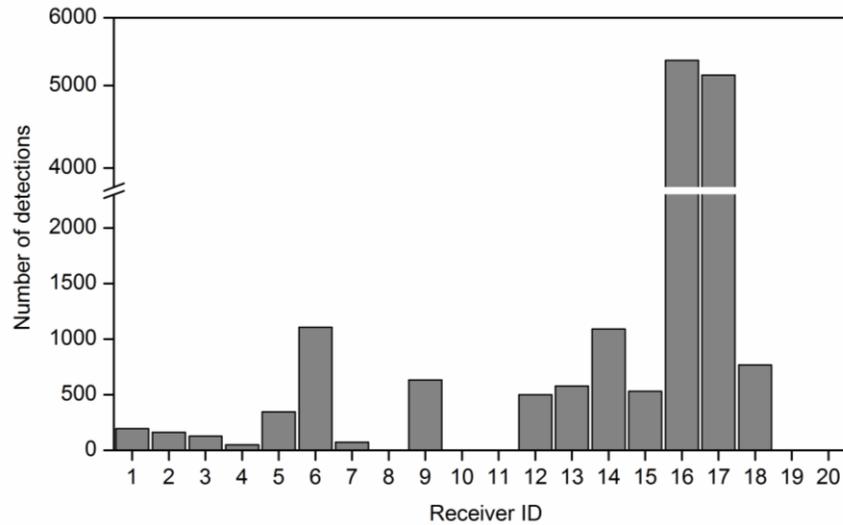
Figure 6.6 Total number of detections from tagged *R. tapirina* recorded on each receiver in the Coorong estuary from May to December 2011.

6.3.3   Movement patterns and capabilities

The spatial distributions of detections of different fish through time were used to describe the movement patterns. Movement patterns were highly variable between individuals (Figure 6.7). Of the two fish tagged near the Murray Mouth (Fish 3754, 3755), one remained within the estuary for 28 hours before exiting through the Murray Mouth and did not return. The other moved between Receivers 2 and 9 over a 20-day period, before leaving the estuary and was not detected again. Of the remaining tagged fish, eight moved from either Receiver 15 or 16 and exited through the Murray Mouth, i.e. distances of 23 km and 31 km respectively (Figure 6.1). Six of these fish did not return and were presumed to have emigrated from the estuary to the sea. Examples of this are Fish 3743 and 3746 (Figure 6.7). One fish (Fish 3740) moved from Receiver 15 and presumably left the estuary, before returning 98 days later (Figure 6.7). Fish 3748 moved from Receiver 15 to the sea and returned to the same receiver seven days later, before repeating this return-migration of at least 46 km, on four other occasions (Figure 6.7). The remaining ten fish that were tagged and released near Receiver 16 moved between Receivers 15, 16 and 17, but did not move from this area. Examples of these are Fish 3751 and 3752. As such, three movement patterns were identified: (1) inner estuary movements; (2) dispersal from the estuary to the sea; and (3) frequent return migrations from the inner estuary to the sea (Figure 6.7).
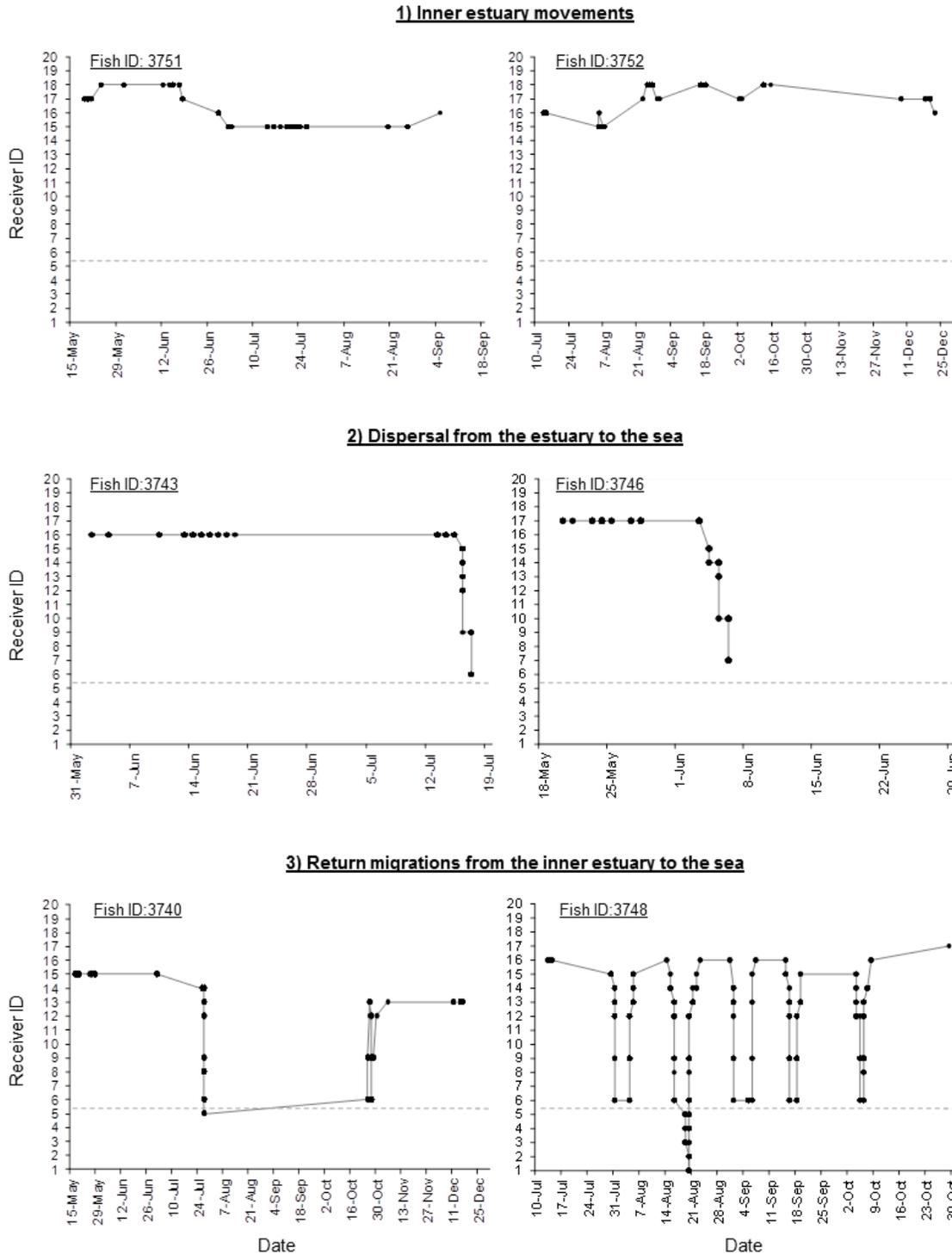
Figure 6.7 Movement patterns of six tagged *R. tapirina* in the Coorong estuary, representing examples of 1) Inner estuary movements; 2) Dispersal from the estuary to the marine environment; and 3) Return migrations from the inner estuary to the sea. Black dots indicate detections and solid grey lines link successive detections but do not necessarily infer direct movement from one receiver to another. The dashed grey line shows the location of the Murray Mouth of the estuary in relation to the receiver array.

Movement activity measured as the number of spatial zones visited per month by individual fish, differed significantly among months (*Pseudo-F$_{7,86}$* = 3.907, *p* = 0.002) (Figure 6.8). Pairwise testing indicated that the number of zones visited in June was significantly higher than all months from August to December, whilst the number of zones visited in July was significantly higher than during November and December (Appendix 6.1).
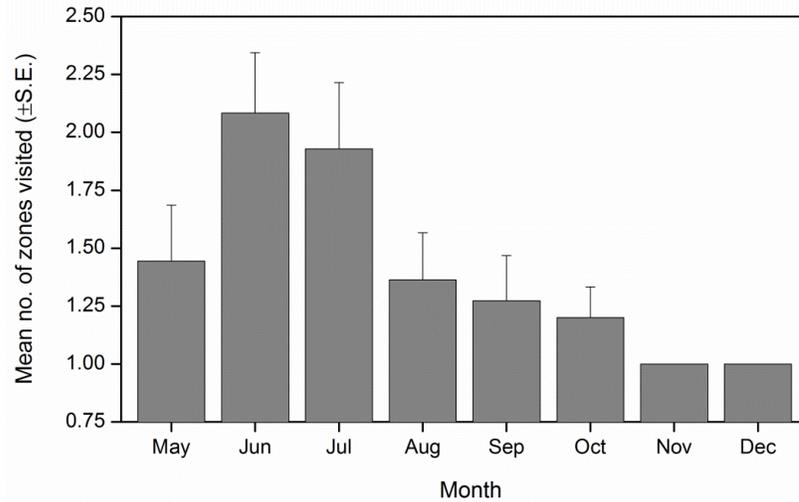
Figure 6.8 Mean numbers of spatial zones visited by *R. tapirina* in the Coorong estuary in each month of the study period.

6.3.4   Residency patterns in relation to salinity

Residency was used to describe the spatial patterns in area use. Tagged fish were recorded in six of the eight zones, as well as at sea, during the study period (Figure 6.9). The interaction effect between zone and month on residence time was not significant (*Pseudo-F$_{56,521}$* = 0.54, *p* = 0.99). Residency varied significantly among zones (*Pseudo-F$_{8,521}$* = 41.3, *p* < 0.001) and this was consistent among months (*Pseudo-F$_{7,521}$* = 0.15, *p* = 0.99). Pairwise analysis indicated that residence time in Zone 6 was significantly higher than for all other zones, whilst residency in Zone 7 was significantly higher than in all other zones, except Zones 5, 6 and the sea (Figure 6.9) (Appendix 6.1). Differences in residency times among all other areas were not significant, as these values were relatively low.
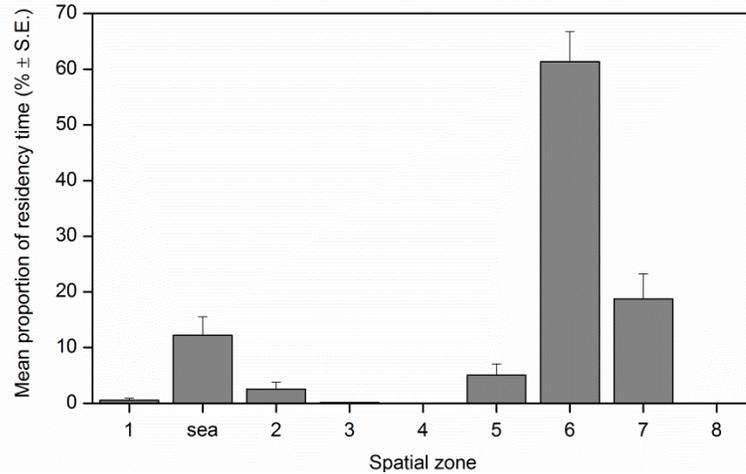
Figure 6.9 Mean proportion of residency time (± S.E.) spent in each spatial zone, including time spent at sea, during the study period.

The relationship between mean daily salinity and residency was examined to help explain spatial variation in area use. Tagged fish occurred in areas with salinities that ranged between 487 µS/cm and 111,815 µS/cm (Figure 6.10). However, observations were not uniformly distributed across this broad range, with fish spending 91% of their time in zones where salinity was less than 60,000 µS/cm. The frequency of observations was highest in salinity intervals between 30,001 and 40,000 µS/cm and between 40,001 and 50,000 µS/cm. In Zone 6, where residence time was highest (Figure 6.9), monthly salinities were more similar to seawater than in other zones (Figure 6.4).
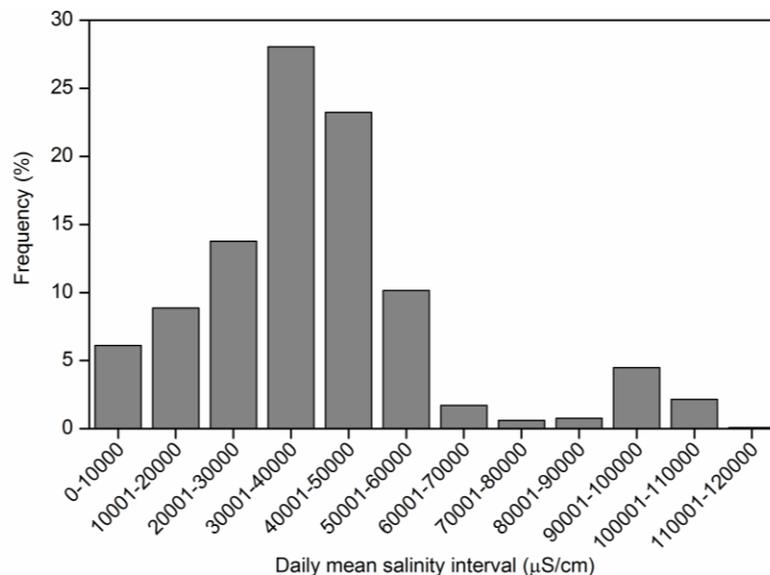
Figure 6.10 Proportion (%) of total residence time spent by *R. tapirina* in specific daily mean salinity intervals (salinity of seawater = 50 000 µS/cm).

6.3.5   Fine-scale variation in habitat use

Estimates of depth from individual detections of tagged *R. tapirina* were used to examine fine-scale patterns in habitat use. Fish were detected across a broad range of depths, from < 0.22 m to 8.13 m, yet 83 % of detections occurred when fish were at depths of between one and three metres (Figure 6.11A).

Habitat-use was examined more closely in the main residency area. In that area, estimates of depth were distributed across a much narrower range compared to other parts of the system, ranging from < 0.22 m to 2.86 m (Figure 6.11B). Significant differences in the depth distributions between day and night indicated a distinct diurnal shift in habitat-use by *R. tapirina* (Kolmogorov-Smirnov $Z = 16.155$, $p < 0.001$). During the day, the depth distribution was bimodal with a large mode at 2 m and a smaller mode at 1 m (Figure 6.11B). In contrast, during the night fish, were commonly found at less than 1.25 m. This diurnal shift was further substantiated by estimates of mean depth at which fish occurred during each hour over the 24-hour period (Figure 6.11C). This time series indicated that fish gradually moved to deeper habitats during the morning, where they remained during the day, before transitioning into shallower water during the late afternoon, where fish remained during the night.

6.4   Discussion

This is the first study to use acoustic telemetry to examine the spatial and temporal patterns of movement, residency and habitat use by the pleuronectid *Rhombosolea tapirina* in an estuarine environment. Despite initial concerns that individual *R. tapirina* may not cope with capture, anaesthetisation and surgery for the implantation of the acoustic transmitters, all fish responded well to the tagging procedure, with most displaying substantial rates of movement throughout the study area. Most movements by fish were confined to the areas southeast of the barrage network, i.e. well away from the freshwater discharge points within the estuary. The areas adjacent to the barrages and Murray Mouth commonly served as a thoroughfare for movement between the inner estuary and the sea. The results of this study challenged the classification of the *R. tapirina* population in the Coorong estuary as an estuarine resident (Hall 1984) and demonstrated the individualistic and often highly transient nature of this species and its ability to undertake regular movements over 10s of kilometres within the estuary and between the estuary and the sea.
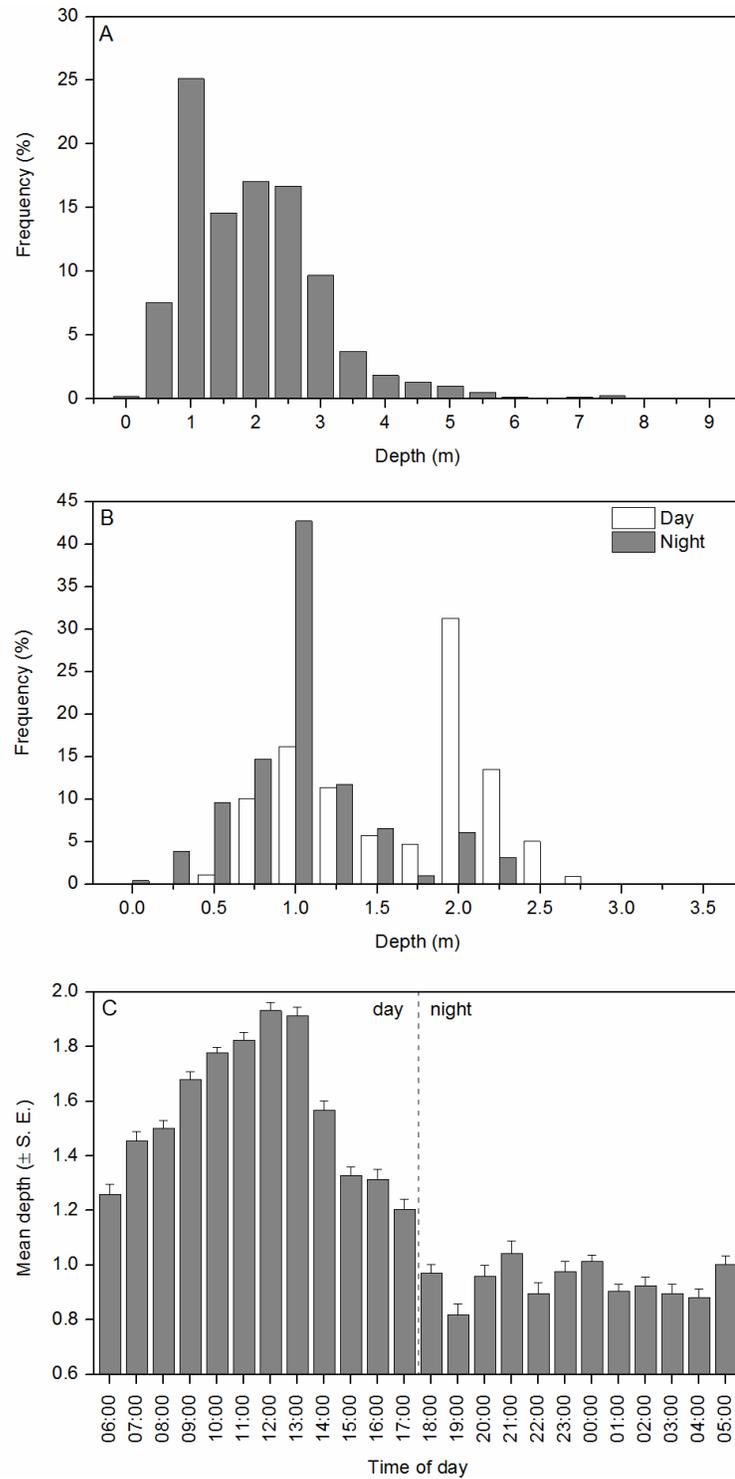
Figure 6.11 (A) – the frequency distribution of depth estimates from detections of *R. tapirina* tagged with acoustic tags in the Coorong estuary; (B) - frequency distribution of depth estimates from detections recorded on Receiver 17; (C) the mean depth of tagged fish, as observed at hourly intervals, based on detections recorded on Receiver 17.

R. tapirina exhibited complex movement patterns that differed considerably among individuals. These were classified into three common patterns: (1) inner estuary movements; (2) dispersal from the estuary to the sea; and (3) frequent return migrations from the inner estuary to the sea. The first movement pattern was consistent with the notion that *R. tapirina* is an estuarine resident (Hall 1984), because individuals in this group remained within the estuary. However, the latter two suggest that the adjacent marine environment plays at least some role in the biology and possible population dynamics of the species and demonstrates the migratory capabilities of *R. tapirina*. Whilst most individuals that emigrated from the estuary did not return, some spent between 1 and 98 days outside the estuary before re-entering and returning to the same area – a round trip of at least 46 km. Movements of this scale have not previously been documented for *R. tapirina*.

The movement of *R. tapirina* between marine and estuarine environments observed in this study is similar to numerous other flatfish species, all of which occur in the northern hemisphere (reviewed in Able and Fahay 1998). However, the behaviour underlying these movements can be quite different among species. For some species, such movements relate to reproduction whereby individuals migrate annually from estuaries to offshore spawning grounds during winter before returning during summer, e.g. *Paralichthys dentatus* (Able and Kaiser 1994; Kraus and Musick 2001), *Pleuronectes platessa* (Rijnsdorp 1990) and *Atheresthes stomias* (Rickey 1995). For *R. tapirina*, a high proportion of the Coorong population spawns inside the estuary during the cooler months of each year (Chapter 4), although the extent of the species' abundance and distribution in the adjacent marine environment is not known. Individuals of other flatfish species emigrate from estuaries as part of an ontogenetic shift in habitat use, whereby juveniles live in estuaries, before migrating to offshore habitats as adults (Szedlmayer and Able 1993). Several studies have also recognized environmental change as a key factor driving fish movement from estuaries to the sea (Armor and Herrgesell 1985; Hedger et al. 2010), although this seems unlikely for *R. tapirina* as this study was undertaken during a period of high freshwater inflow – a time when population abundance is often highest (see Chapter 2). At present, it is not possible to determine the reason for the offshore migrations undertaken by *R. tapirina* from the Coorong estuary. Nor is it possible to estimate the extent of immigration to the estuary from other populations and how that may affect population size. Such information is imperative for understanding the processes that contribute to temporal fluctuations in population abundance.

Although the present study was relatively short, there was evidence of seasonal variation in movement activity in 2011, with relatively high activity in June and July compared to the other months. The timing of this increased activity occurred when the spawning frequency was highest (Chapter 4), suggesting that it may have been associated with reproductive activity.

Despite the diverse and complex movement patterns exhibited by *R. tapirina*, the residency was generally limited to the inner estuary. On average, tagged fish spent approximately 80 % of their time residing in this area, mostly in Zone 6. Residence times adjacent to the barrages and near the Murray Mouth were generally low and reflected the time fish spent moving between the inner estuary and the sea, rather than prolonged occupancy in these areas. Among the numerous individuals that undertook seaward migrations from the main area of residence, two fish re-entered the system, and in both instances, returned to the inner estuary. Furthermore, of the twenty fish tagged, 50 % were permanent residents within the inner estuary for the duration of the study. This suggests that environmental conditions in this area were favourable for the species.

Salinity is commonly viewed as one of the most important environmental variables that influence the distribution of fish within estuaries (Marshall and Elliott 1998). Here, the distribution and residency patterns of *R. tapirina* in the Coorong estuary reflected a clear preference for a particular salinity range. Whilst individuals were recorded across a broad range of salinities, tagged fish spent longer in areas characterised by brackish and near-marine conditions, i.e. the mesohaline zone of the estuary. For the duration of the present study, the mesohaline zone occurred in the inner estuary, where hypersaline waters of the southern Coorong merged with freshwater discharged through the barrages. In other estuaries, this transitional zone is known to support higher primary productivity relative to other areas of the estuary during high flow events (Boyer et al. 1993), which may explain *R. tapirina*'s preference for these conditions. Such a well-defined salinity preference is characteristic of numerous other flatfish species that inhabit estuaries, e.g. *Paralichthys lethostigma* (Allen and Baltz 1997; Walsh et al. 1999) and *P. dentatus* (Powell and Schwartz 1977; Gibson 1994).

Diel period can also be a strong determinant of fish movement and spatial behaviour (reviewed in Gibson 1997). Tagged *R. tapirina* exhibited a distinct diurnal shift in fine-scale habitat use in the Coorong estuary, with individuals occupying deeper habitats, such as holes and channels, during the day, before moving up to shallower habitats, such as mudflats and sandbars, during the night. This behaviour is most likely driven by the search for prey in the food-rich flats and/or in the

water column of the inner Coorong estuary during the hours of darkness, as reported for other flatfish species including *Pleuronectes platessa* (Burrows et al. 1994), *Platichthys flesus* (Wirjoatmodjo and Pitcher 1984) and *Solea senegalensis* (Vinagre et al. 2006). Avoidance of visual predators may also be an important function of nocturnal movement into shallower water. Flatfishes characteristically limit their activity during the day and often bury in the sediment to reduce predation risk (Gibson 2005). Nocturnal movements of *R. tapirina* into shallower habitats may reduce the risk of predation by visual predators such as New Zealand fur seals and large-bodied fish species such as mulloway (*Argyrosomus japonicus),* both of which are common in the Coorong estuary (Shaughnessy et al. 2012; Ferguson et al. 2013).

This study has demonstrated the individualistic and highly transient nature of *R. tapirina* behaviour and has contributed important information on the potential role of movement in its population dynamics. The emigration of some individuals from the estuary to the sea suggests that truncated age structure of the Coorong population, as determined in Chapter 3, may relate to the movement of older fish. Furthermore, the species' clear preference for brackish and marine-like conditions indicates that the previously documented variation in population abundance in the system is likely driven by variation in the amount of estuarine habitat available for the species. This is likely to contribute to inter-annual variation in egg production and recruitment within this system. Development of management strategies for this population of *R. tapirina* need to consider migration as a key demographic process that may influence stock structure and population abundance.

7 General Discussion

The overall aim of this study was to inform on the population biology and ecology of the greenback flounder, *Rhombosolea tapirina*, in the Coorong estuary, South Australia, and the factors and processes that regulate population structure and abundance. In particular, this research considered some spatial aspects of the adult biology of the species in the context of significant environmental changes that occurred in mid-2010. Specific investigations focused on: long-term patterns of population abundance and dispersion in relation to river discharge; age, growth and population structure; reproduction; feeding ecology and trophic relationships; and movement, residency and fine-scale habitat use. This chapter summarises the key findings of these different investigations and discusses their collective implications with respect to the variable population abundance of *R. tapirina*. Furthermore, the management implications of the findings are considered along with recommendations for future research.

7.1 Overview of results

7.1.1 Historical patterns of population abundance

Analysis of the long-term chronology of the fishery production of *R. tapirina* in the Coorong estuary indicated extreme inter-annual variation in population abundance and dispersion. Commercial catches ranged from > 230 t.yr^{-1} in the mid-1970s, to < 1 t.yr^{-1} in the late 2000s. Furthermore, the population sizes were related to the area of the estuary over which the animals were dispersed. This variation in abundance and extent of dispersion were driven partly by fluctuations in the magnitude of freshwater inflow to the system, because large areas of estuarine habitat that support high abundances of *R. tapirina* are only available during and immediately after years of increased inflow. The long-term periodic variation in population abundance suggests the likelihood that high fishing mortality experienced in the peak fishing years further affected the dynamics of the population. Such flow-related variation in estuarine fish abundances and fishery catches has been reported previously for numerous fish species (Loneragan and Bunn 1999; Blaber et al. 2000; Gillson et al. 2009), including mulloway (*Argyrosomus japonicus*) in the Coorong estuary (Ferguson et al. 2008).

7.1.2   Age, growth and population structure

A direct fish ageing protocol based on interpreting the macrostructure of otoliths of *R. tapirina* was developed and provided estimates of age-based data. Male and female fish grew at the same rapid rate and achieved approximately 65 % of their asymptotic length after just 12 months of life. Such fast growth is characteristic of all species in the Australasian flatfish genus *Rhombosolea* (Coleman 1974; Francis 1988a; Paul 1992). Despite the potential for this species to achieve a maximum age of 10 years (Sutton et al. 2010), the population in the Coorong estuary was dominated by 1+ and 2+ fish, while older fish were rare. As such, this population is considered truncated in age, with minimal biomass stored in fish of 3 years of age and older.

7.1.3   Reproductive biology

Assessment of the reproductive biology of *R. tapirina* in the Coorong indicated that the species is a multiple batch spawner, with asynchronous oocyte development and indeterminate fecundity. This is consistent with that for other populations of *R. tapirina* of south-eastern Australia (Kurth 1957; Barnett and Pankhurst 1999). These characteristics enable individuals to spawn on numerous occasions throughout the prolonged spawning season. There was a distinct seasonal trend in reproductive development and spawning activity, i.e. spawning occurred from March to October, and was most frequent from May to August, i.e. the months of the year when water temperature was lowest. Comparison of the reproductive biology between areas of contrasting salinity regimes showed strong similarities in spawning activity. This suggests that either mixing occurred between the two areas, or that differences in salinity did not affect the physiological processes that lead to gonad development and then to the maturation of oocytes. Male and female *R. tapirina* attained sexual maturity at approximately 13 and 14 months, respectively, i.e. on average, they became reproductively mature in the winter after they were spawned.

7.1.4   Feeding ecology and trophic relationships

R. tapirina, like numerous other flatfish species, is a carnivorous, generalist feeder that exploits food resources from a wide array of taxonomic groups (Link et al. 2005). The main components of the diet were polychaetes, gammarid amphipods, and bivalve siphons, whilst other small crustaceans, annelids and insect larvae were also consumed in relatively low amounts. *R. tapirina* exhibited a distinct ontogenetic trophic shift that likely related to changes in feeding morphology. Spatial differences in diet were marginal, whilst there was no clear seasonality in feeding habits. In contrast, there were distinct differences in diet before and after the commencement of drought-

breaking freshwater inflows which likely related to changes in prey availability (Edgar and Shaw 1995).

7.1.5   *Movement, residency and habitat use*

Analysis of the movement patterns of adult *R. tapirina* during a period of relatively high freshwater discharge demonstrated the individualistic and often highly transient nature of the movement behaviour of the species. Tagged fish were detected over a large part of the system, from near the Murray Mouth to approximately 45 km away in the inner estuary. These movement patterns were identified as: (1) inner estuary movements; (2) dispersal from the estuary to the sea; and (3) frequent return migrations over 10s of kilometres from the inner estuary to the sea. Tagged fish demonstrated a clear preference for 'estuarine habitat' characterised by brackish and marine-like conditions. Furthermore, they exhibited a distinct diurnal shift in fine-scale habitat use, with individuals occupying deeper habitats, such as holes and channels, during the day, before they moved up to shallower habitats, such as mudflats and sandbars, during the night. Such behaviour likely relates to nocturnal foraging behaviour, a possible adaptation to minimise the risk of predation (Gibson 2005).

7.2   **Life history of *R. tapirina* in the Coorong estuary**

R. tapirina is an important component of the fish assemblage in the Coorong estuary, South Australia (Ferguson et al. 2013). This study provided important information on the adult biology of species in the Coorong, as well as the factors and processes that regulate its population abundance. The findings of this research enable the development of a qualitative model of the life history of the species in this environmentally-complex system.

A significant finding of this study was that the age structures of *R. tapirina* in the Coorong were restricted to a few young age classes. Given the potential of this species to reach ten years of age, this indicates that this population was truncated. Such truncation related the removal of larger, older individuals by the commercial fishery, as well as an ontogenetic migration from the estuarine to marine environment that occurred during the second or third years of life. The seaward movement of fish from the estuary suggests that the adult biomass of the population is not confined to the estuary. Whilst the extent of the offshore part of the population is not known, the older fish from the Coorong are likely to move a considerable distance offshore, as the extensive high-energy surf beaches immediately adjacent to the estuary mouth are unlikely to

provide suitable habitat for *R. tapirina.* In other parts of the species' distribution, individuals have been recorded in offshore waters in depths of up to 100 m (Gomon et al. 2008).

Although the Coorong does not appear to support individuals of *R. tapirina* throughout their adult lives, the estuary provides an important nursery area for the species through the first 2-3 years of life. In the estuary, post-larvae and small juveniles inhabit shallow, unvegetated sandy habitats from winter through to summer in each year (Ye et al. 2011, Livore et al. 2013). During their first 12 months, individuals grew rapidly and reached ~225 mm in total length, i.e. approximately 65 % of the asymptotic length of the species. Both male and female fish reached sexually maturity early in their second year, which enabled them to spawn in the winter following when they themselves were spawned. Despite a strong bias in numbers toward females in the population, spawning occurred within the estuary and involved the release of multiple batches of eggs over a protracted period from March to October in each year, and was most frequent when water temperatures were below 14 $^{\circ}$C. Whilst the estuarine spawning biomass is likely to contribute to localised recruitment within the system, the contribution of recruits that originate from any spawning events in the marine environment outside the estuary remains unknown.

The abundance and dispersion of juvenile and adult *R. tapirina* in the estuary were strongly influenced by the amount of estuarine habitat available within the system, which is regulated through seasonal inflows of freshwater (Livore et al. 2013; Webster 2010). Under a regime of consistent seasonal flow, extensive areas of estuarine habitat are available and commonly support high densities of *R. tapirina.* Alternatively, during prolonged periods of no flow, population abundance is generally very low and the dispersion of individuals is restricted to the vicinity of the Murray Mouth, because the majority of the Coorong becomes hypersaline. It is possible that such spatial contractions of the population within the estuary force some *R. tapirina* into the marine environment, where they remain and can possibly return when estuarine conditions improve. An example of this occurred recently when commercial catches of *R. tapirina* increased from < 2 t.yr^{-1} in the final years of the Millennium Drought (2008/09 to 2010/11), to > 31 t in 2011/12, after several months of unregulated high inflows to the estuary. The abrupt increase in catch related to the movement of mostly two year old fish back into the estuary from the marine environment. As recruitment of *R. tapirina* within the Coorong in the 3-4 years prior to this event was minimal (Livore et al. 2013), it is likely that the majority of the fish landed by the fishery in 2011/12, were spawned and spent the first two years of their life in the marine environment. Flow-related increases in population abundance highlighted: (1) the strong relationship between

the amount of estuarine habitat available and population abundance for *R. tapirina* in the Coorong estuary; (2) the opportunistic nature of the species' use of the Coorong; (3) the movement capabilities and sensitivity of the species to environmental changes within the estuary; (4) the ability of the species to live in the marine environment if required; and (5) the potential for a large biomass of adult fish to move into the estuary when environmental conditions are suitable.

Information on the life history characteristics and adult biology of *R. tapirina* in the Coorong estuary was considered to classify the species to one of the 14 functional guilds for estuarine fish defined by Elliott et al. (2007). Despite some uncertainty regarding the role of the marine environment immediately adjacent to the Coorong estuary in the life-history of *R. tapirina*, knowledge compiled in this research project suggests that the species fits into the sub-guild of 'marine migrants', known as 'marine estuarine-opportunists'. This group of fishes comprises marine species that enter estuaries in substantial numbers, particularly during the juvenile and early adult stages, but use, to varying degrees, nearshore marine waters as an alternative habitat (Elliott et al. 2007). This challenges the classification of the species by Hall (1984), who concluded that *R. tapirina* was an 'estuarine resident'.

7.3   Implications for management and considerations for future research

The findings of this research indicated that the previously documented variation in fishery production for *R. tapirina* is likely related to a combination of anthropogenic and demographic events and/or processes that occur during the first 2-3 years of its life history. The most significant of which involves: the movement of individuals from the estuary to the marine environment during their second or third year of life; fluctuations in the amount of estuarine habitat available within the Coorong as a consequence of extreme variability in freshwater inflows to the system; and the removal of individuals by the commercial fishery. The sustainable management of the *R. tapirina* population in the Coorong estuary would depend on an ecosystem-based management approach that would seek to:

- provide a regime of consistent seasonal freshwater inflow to restore and maintain extensive areas of estuarine habitat that can support high densities of *R. tapirina* through at least the first 2-3 years of its life history;

- maintain connectivity between the Coorong estuary and the marine environment to provide fish passage and accommodate the opportunistic use of the system by the species; and

- provide protection for the environmentally-mediated dispersion and abundance of the *R. tapirina* population in the Coorong from commercial fishing, particularly during periods of poor environmental condition, to ensure the species is harvested sustainably.

To prioritise management action for exploited estuarine fish populations, there is a need for knowledge of stock structure and the degree to which exploited stocks are limited by recruitment (Peterson and Summerson 1992). Future research on *R. tapirina* in South Australia should seek to provide a better understanding of the role of early life history events in the population biology of the species in the Coorong, as well as over broader spatial scales. The issue of spatial scale is crucial for understanding stock-recruitment relationships (Begg and Waldman 1999), and the capacity to which populations can be replenished if localised recruitment does not occur. Furthermore, given the regular movements of individual *R. tapirina* between the estuarine and marine environment documented in this study, future research should also seek to better understand the connectivity between populations along the southern Australian coast using modern molecular techniques and otolith chemistry. Until such information is available, a precautionary approach in managing the population is required.

Appendices

Appendix 5.1 SIMPER analysis for pair-wise comparisons of dietary composition between size classes of *R. tapirina* for the two study areas in the Coorong estuary. Results are based on square root transformed data. Mean contribution is the average volumetric contribution of each prey items to the diet. Contrib% = percentage contribution to the difference between seasons. A cumulative cut-off of 60 % was applied. Mean dissimilarity expressed as a percentage ranging between 0 % (identical) and 100 % (totally dissimilar).

Study area	Species	Mean contribution		Contrib.%	Cum.%
Estuary		**small**	**medium/large**	Mean dissimilarity = 73.8	
	Nephtys australiensis	0.09	0.5	20.46	20.46
	Amphipoda: Gammaridea	0.66	0.35	20.34	40.80
	bivalve siphon	0.22	0.18	11.7	52.50
	Copepoda	0.27	0	11.18	63.68
North Coorong		**small**	**medium/large**	Mean dissimilarity = 78.9	
	Amphipoda: Gammaridea	0.69	0.29	20.83	20.83
	Nephtys australiensis	0.03	0.42	16.54	37.38
	bivalve siphon	0.07	0.31	13.05	50.43
	unidentified material	0.25	0.09	10.39	60.81

Appendix 5.2 SIMPER analysis for pair-wise comparisons of dietary composition between study areas for each size class of *R. tapirina*. Results are based on square root transformed data. Mean contribution is the average volumetric contribution of each prey items to the diet. Contrib% = percentage contribution to the difference between seasons. A cumulative cut-off of 60 % was applied. Mean dissimilarity expressed as a percentage ranging between 0 % (identical) and 100 % (totally dissimilar).

Size class	Species	Mean contribution		Contrib.%	Cum.%
small		**North Coorong**	**Estuary**	Mean dissimilarity = 53.9	
	Copepoda	0.15	0.27	15.13	15.13
	unidentified material	0.25	0.19	14.23	29.35
	Amphipoda: Gammaridea	0.69	0.66	13.48	42.84
	bivalve siphon	0.23	0.22	13	55.84
	Mysidacea	0.1	0.13	9.77	65.60
medium/large		**North Coorong**	**Estuary**	Mean dissimilarity = 69.1	
	Nephtys australiensis	0.42	0.5	21.91	21.91
	Amphipoda: Gammaridea	0.29	0.35	19.43	41.34
	bivalve siphon	0.31	0.18	17.15	58.49
	Amphipoda (*Melita* sp.)	0.09	0.07	6.52	65.01

Appendix 5.3 SIMPER analysis for pair-wise comparisons of dietary composition between seasons for each size class of *R. tapirina* in the Estuary. Results are based on square root transformed data. Mean contribution is the average volumetric contribution of each prey items to the diet. Contrib% = percentage contribution to the difference between seasons. A cumulative cut-off of 60 % was applied. Mean dissimilarity expressed as a percentage ranging between 0 % (identical) and 100 % (totally dissimilar).

Size class	Species	Mean contribution		Contrib.%	Cum.%
		summer	**winter**	Mean dissimilarity = 54.6	
small	*Nephtys australiensis*	0.08	0.39	19.85	19.85
	Amphipoda: Gammaridea	0.73	0.5	18.49	38.35
	bivalve siphon	0.42	0.32	15.96	54.3
	Copepoda	0.03	0.28	14.48	68.79
		summer	**spring**	Mean dissimilarity = 58.8	
	Copepoda	0.03	0.48	24.41	24.41
	bivalve siphon	0.42	0	21.46	45.88
	Mysidacea	0.01	0.28	14.41	60.29
		winter	**spring**	Mean dissimilarity = 58.9	
	Nephtys australiensis	0.39	0	17.9	17.9
	Amphipoda: Gammaridea	0.28	0.48	14.56	32.46
	Copepoda	0.32	0	14.44	46.91
	bivalve siphon	0.5	0.63	13.73	60.64
		summer	**winter**	Mean dissimilarity = 60.5	
medium & large	*Nephtys australiensis*	0.74	0.53	21.88	21.88
	Amphipoda: Gammaridea	0.1	0.33	16.44	38.32
	bivalve siphon	0.21	0.25	15.83	54.15
	Amphipoda (*Melita* sp.)	0	0.15	8.15	62.29
		summer	**spring**	Mean dissimilarity = 80.5	
	Amphipoda: Gammaridea	0.1	0.76	30.81	30.81
	Nephtys australiensis	0.74	0.2	27.59	58.4
	bivalve siphon	0.21	0.05	8.99	67.4
		autumn	**spring**	Mean dissimilarity = 76.6	
	Amphipoda: Gammaridea	0.19	0.76	28.91	28.91
	Nephtys australiensis	0.57	0.20	22.45	51.36
	bivalve siphon	0.19	0.05	9.14	60.49
		winter	**spring**	Mean dissimilarity = 68.5	
	Amphipoda: Gammaridea	0.33	0.76	26.18	29.18
	Nephtys australiensis	0.53	0.2	22.89	49.07
	bivalve siphon	0.25	0.05	11.71	60.78

Appendix 5.4 SIMPER analysis for pair-wise comparisons of dietary composition between seasons for each size class of *R. tapirina* in North Coorong. Results are based on square root transformed data. Mean contribution is the average volumetric contribution of each prey items to the diet. Contrib% = percentage contribution to the difference between seasons. A cumulative cut-off of 60 % was applied. Mean dissimilarity expressed as a percentage ranging between 0 % (identical) and 100 % (totally dissimilar).

Size class	Species	Mean contribution		Contrib.%	Cum.%
		summer	**winter**	Mean dissimilarity = 53.3	
small	bivalve siphon	0.42	0.18	20.88	20.88
	unidentified material	0.13	0.32	16.81	37.69
	Amphipoda: Gammaridea	0.73	0.63	15.06	52.75
	Copepoda	0.03	0.17	9.86	62.6
		summer	**spring**	Mean dissimilarity = 55.2	
	bivalve siphon	0.42	0.01	22.71	22.71
	unidentified material	0.13	0.21	13.01	35.73
	Amphipoda: Gammaridea	0.73	0.73	12.84	48.57
	Copepoda	0.03	0.13	7.79	56.36
	Mysidacea	0.01	0.13	7.52	63.88
		summer	**winter**	Mean dissimilarity = 55.8	
medium & large	*Nephtys australiensis*	0.38	0.46	20.99	20.99
	Amphipoda: Gammaridea	0.11	0.4	18.24	39.23
	bivalve siphon	0.22	0.3	17.46	56.69
	Paragrapsus gaimardii	0.2	0.01	9.21	65.9
		summer	**spring**	Mean dissimilarity = 87.2	
	Amphipoda: Gammaridea	0.11	0.76	26.86	26.86
	Nephtys australiensis	0.38	0	15.14	42
	bivalve siphon	0.22	0.19	11.89	53.89
	Amphipoda (*Melita* sp.)	0.02	0.28	10.52	64.41
		autumn	**winter**	Mean dissimilarity = 64.9	
	Nephtys australiensis	0.57	0.46	22.42	22.42
	bivalve siphon	0.39	0.3	22.09	44.51
	Amphipoda: Gammaridea	0.06	0.4	19.25	63.76
		autumn	**spring**	Mean dissimilarity = 88	
	Amphipoda: Gammaridea	0.06	0.76	27.14	27.14
	Nephtys australiensis	0.57	0	21.31	48.45
	bivalve siphon	0.39	0.19	15.38	63.83
		winter	**spring**	Mean dissimilarity = 69.6	
	Amphipoda: Gammaridea	0.4	0.76	23.49	23.49
	Nephtys australiensis	0.46	0	22.26	45.75
	bivalve siphon	0.3	0.19	17.5	63.25

Appendix 6.1 Results of PERMANOVA pairwise analyses comparing the mean number of spatial zones visited by *R. tapirina* in the Coorong estuary between months (α= 0.05).

Pairwise comparisons between months	t	P	Unique permutations
August, December	1.75	0.21	6
August, July	1.48	0.20	57
August, June	**2.85**	**0.05**	**38**
August, May	0.26	0.98	15
August, November	1.75	0.21	6
August, October	0.61	0.72	10
August, September	0.35	0.93	6
December, July	**2.81**	**0.02**	**30**
December, June	**3.91**	**0.00**	**24**
December, May	1.99	0.09	6
December, November	no test		
December, October	1.50	0.47	2
December, September	1.36	0.47	4
July, June	0.49	0.60	89
July, May	1.13	0.29	55
July, November	**2.81**	**0.02**	**30**
July, October	1.99	0.07	49
July, September	1.79	0.10	48
June, May	1.73	0.13	35
June, November	**3.91**	**0.00**	**24**
June, October	**2.81**	**0.02**	**33**
June, September	**2.51**	**0.03**	**34**
May, November	1.99	0.08	6
May, October	0.86	0.47	10
May, September	0.60	0.71	12
November, October	1.50	0.48	2
November, September	1.36	0.47	4
October, September	0.23	1.00	8

Appendix 6.2 Results of PERMANOVA pairwise analyses comparing the residency time for *R. tapirina* in the Coorong estuary between spatial zones ($\alpha = 0.05$).

Pairwise comparisons between spatial zones	t	P	Unique permutations
8, 7	**2.89**	**0.02**	**9708**
8, 6	**9.55**	**0.00**	**9791**
8, 5	1.85	0.08	9954
8, 4	no test		
8, 3	0.87	0.57	93
8, 2	1.12	0.38	9931
8, SEA	2.36	0.06	9655
8, 1	0.95	0.56	93
7, 6	**5.71**	**0.00**	**9844**
7, 5	1.36	0.22	9828
7, 4	**2.89**	**0.03**	**9677**
7, 3	**2.87**	**0.03**	**9773**
7, 2	**2.55**	**0.05**	**9842**
7, SEA	0.99	0.36	9863
7, 1	**2.81**	**0.03**	**9827**
6, 5	**8.56**	**0.00**	**9823**
6, 4	**9.55**	**0.00**	**9782**
6, 3	**9.52**	**0.00**	**9814**
6, 2	**9.13**	**0.00**	**9806**
6, SEA	**6.67**	**0.00**	**9835**
6, 1	**9.40**	**0.00**	**9816**
5, 4	1.85	0.08	9950
5, 3	1.83	0.08	9957
5, 2	1.29	0.26	9961
5, SEA	0.59	0.59	9854
5, 1	1.71	0.10	9959
4, 3	0.87	0.58	93
4, 2	1.12	0.37	9929
4, SEA	2.36	0.05	9722
4, 1	0.95	0.56	93
3, 2	1.06	0.42	9958
3, SEA	2.34	0.06	9703
3, 1	0.74	0.69	6907
2, SEA	1.89	0.11	9861
2, 1	0.75	0.59	9935
SEA, 1	2.33	0.06	9735

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