EVALUATING THE IMPORTANCE OF MANGROVES AS FISH NURSERIES IN SELECTED WARM TEMPERATE SOUTH AFRICAN ESTUARIES

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GENERAL ABSTRACT

The value of mangrove habitats as fish nurseries was assessed by comparing communities of early stage and juvenile fishes between estuaries with and without mangroves. Early stage fishes were sampled using boat-based plankton towing while juveniles were sampled by seine netting. Sampling took place at five sites spaced 1 km apart starting near the estuary mouth in four estuaries along the temperate coastline of the Eastern Cape Province of South Africa. Four estuaries were selected based on shared similarities which included catchment area, estuarine area and shared habitats barring the presence of mangroves which occupied the river margins of two systems. Results revealed that early stage and juvenile fish communities (both marine- and estuary-spawned) were similar between systems with and without mangrove habitats. Differences in fish communities among estuaries were rather attributed to axial salinity gradients associated with greater freshwater input, while season and temperature produced significant variances in fish densities with Generalised Additive Models revealing responses of communities to these variables. A common estuarine-dependent fish, *Rhabdosargus holubi* (Family Sparidae), was further investigated to determine habitat use, residency and dietary patterns in different mangrove habitats. High habitat residency in this species was revealed during a short-term tagging study using Visible Implant Elastomer tags and long-term isotope analysis in juveniles sampled from two contrasting mangrove habitats. A wider feeding niche was observed in an eelgrass-red mangrove connected habitat when compared with more exposed white mangrove areas. Low dependence on mangrove habitats in temperate estuaries is likely due to their tidally dominated inundation and limited refuge potential due to smaller area coverage by mangroves in temperate estuaries. Relatively lower primary productivity in warm temperate mangrove areas, relative to their tropical counterparts, provides no significant feeding advantage or refuge opportunities relative to other available habitats in these estuaries. Warm temperate estuaries, which are both spatially and temporally highly variable, instead host species which are habitat generalists, able to capitalise on these highly dynamic environments.

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DECLARATION

I, Cuen Muller (20232739), hereby declare that the thesis for Master of Science is my own work and that it has not previously been submitted for assessment or completion of any postgraduate qualification to another University or for another qualification.

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CHAPTER 1

GENERAL INTRODUCTION

1.1. CONCEPTUAL DEVELOPMENT OF FISH NURSERY DEFINITION

The nursery role of shallow estuarine and coastal waters has been a long-standing concept proposed through observations of high abundances for many species of juvenile fishes in these areas as well as the spatial separation of juvenile and adult populations (Boehlert and Mundy 1988; Blaber and Blaber 1980; Mulkana 1966). Early studies revealed this spatial separation of marine spawned organisms, in which early-life stages migrate to or are transported to estuaries, grow to subadults, and then join offshore adult populations suggesting that these areas may provide critical habitat in supporting fishery stocks (Mulkana 1966; Hay 1905). Originally, the concept proposed that entire estuaries were nurseries, which may have been due to the preceding qualitative observations but also due to a lack of a clear testable nursery-habitat definition (Heck et al. 2003; Beck et al. 2001). Thayer et al. (1978) was among the first to provide a definition and proposed that a nursery habitat must provide protection from predators, or a varied and concentrated food source (Sheridan and Hays 2003). Later definitions built upon this outline until Beck et al. (2001) provided a clear and testable definition which states that a habitat is a nursery for a given species if it contributes disproportionately per unit area to the production of individuals which successfully recruit to adult habitats. This is brought about by a combination of four factors where a given habitat may either support greater (1) densities, (2) growth, (3) survival of juveniles, and/or (4) movement to adult habitats (Beck et al. 2001). Dahlgren et al. (2006) extended this definition by adding the Effective Juvenile Habitat concept which recognizes that some nursery habitats, based on their restricted spatial extent, are of relatively lower conservation value when compared to larger areas where the densities of organisms may be less.

More recently, several studies have noted the importance of habitat mosaics and have attributed greater abundances of juveniles and adult populations to the functional connectivity of habitat patches (Sheaves et al. 2015; Jelbart et al. 2007; Mumby et al. 2004). For example, Nagelkerken et al. (2001) noted that embayment's with both seagrass and mangrove habitats had greater species diversity and densities than bays with only seagrass. This spatial connectivity of habitat patches has been termed the 'seascape nursery' by Nagelkerken et al. (2015) who criticises the approach of valuing nurseries as static, isolated habitats.

1.2. OVERVIEW OF RESEARCH ON MANGROVES AS FISH HABITATS

Selection for specific habitats at the start of the fish life cycle has been shown to be an active process and one which will affect competitive interactions, food availability and survival with knock-on effects into the juvenile stage (Levin et al. 1997; Bell and Westoby 1986). Subsequently, habitats which provide structural heterogeneity typically harbour higher densities of fishes than alternate structurally less complex habitats (Nagelkerken 2009; Heck et al. 2003; Heck and Orth 1980). This is generally attributed to three mechanisms comprising structural heterogeneity, food availability and refuge from predation (Nagelkerken and Faunce 2008; Adams et al. 2004; Laegdsgaard and Johnson 2001). The roots of mangrove trees are often found to provide a structural habitat where food availability and refuge are higher than adjacent habitats. Mangroves occur throughout the tropics and are prominent in shallow coastal systems such as bays and estuaries. The concomitant occurrence of juveniles of many economically important species within these habitats has resulted in much research on the value of these habitats to coastal fish populations (Hogarth 2015; Nagelkerken 2009; Faunce and Serafy 2006). Experimental testing by both Laegdsgaard and Johnson (2001) and Nagelkerken and Faunce (2008) have shown separately that artificial mangrove structure, particularly with epiphytic growth, attracts more species than alternate non-structural habitat. Some species appeared to be attracted to the structural component, being present when structure was clean of epiphytic growth as well as free from predators, while further species were attracted when piscivorous fishes were included or structure allowed to accumulate algal growth indicating a dependency on refuge and food provision, respectively (Laegdsgaard and Johnson 2001). Mangrove roots are also sites of active sediment accretion and deposition where loose sediment and high organic content result in higher densities of meiofauna and meroplankton than adjacent areas (Zhou 2001).

While many tropical studies have substantiated the value of mangroves as important nursery areas others have not found any evidence of increased value relative to alternate habitats. Faunce and Serafy (2006) reviewed studies on fish-mangrove relationships concluding that the results of any study can be either bolstered or refuted with the current available literature. Despite this, a dogma exists where the presence of mangroves is associated with high densities of juvenile fishes. This view is primary to the conservation of mangroves worldwide (Alongi 2002) and has been extended to those occurring in warm temperate waters despite an absence of research from these climates (Morrisey et al. 2010). Of the 111 papers reviewed by Faunce and Serafy (2006) only one had originated from temperate climates. More recently, however, there has been an increase in studies from south Australia and New Zealand assessing the relationship between fishes and warm temperate mangroves with mixed findings (Payne and Gillanders 2009; Bloomfield and Gillanders 2005; Smith and Hindell 2005).

1.3. RATIONALE

While there are many studies comparing densities or communities of fishes amongst individual habitats to determine variable habitat value (e.g. Faunce and Serafy 2006, for review), the limited spatial extent of these studies may not be suitably representative of features such as mangroves. Even though mangroves are predicted to provide local advantages specific to their root systems, such as a structural habitat where feeding and refuge are greater, they also provide autochthonous nutrient inputs through leaf litter as significant primary producers in many estuarine systems (Kathiresan and Bingham 2001).

Similarly, the roots of mangroves provide regions of accretion where loose sediment aggregates and is rich with meio- and macro-fauna, such as harpacticoid copepods and crabs, which contribute significantly to the cyclic production of meroplankton through their larvae (Nagelkerken et al. 2008; Kathiresan and Bingham 2001). These habitat-specific features therefore have wide-ranging impacts and benefits for the greater ecological system. Nevertheless, the majority of studies compare habitats within ecosystems and as a result "studies are undertaken at scales very different from those that are most relevant to the ecological phenomena under study" (Pittman and McAlpine 2003). A key factor is therefore to identify and undertake studies at both small and large scales which can evaluate animal-environment relationships (Faunce and Layman 2009; Pittman and McAlpine 2003). For a thorough assessment of the contribution of mangroves to fish communities, comparisons should therefore encompass the greater ecological area such as an estuary or bay and not be specific to their immediate habitats alone.

1.4. AIMS AND OBJECTIVES

While mangroves are dominant features of tropical coastlines the distribution of some mangrove species extends into warm temperate climates where their value as fish nurseries has often been uncritically applied (Morrisey et al. 2010). However, temperate mangrove forests differ from tropical systems in that they are far less diverse, have lower structural complexity, smaller pool of associated organisms and have lower rates of primary production (Morrisey et al. 2010). Nevertheless, the presence of mangroves, as an added habitat niche, may well be associated with a more diverse fish assemblage as several studies in South African estuaries have noted that estuaries with diverse habitats for fishes often have higher fish species diversity than more uniform systems (Strydom 2015; Whitfield 1999).

The main aim of this research was to determine the importance of mangroves for larval and juvenile fish communities in estuaries along the temperate, south-eastern coastline of South Africa. Additionally, a range of collected temporal and spatial environmental factors were used to investigate trends in associated species richness and abundance of marine- and estuary-spawned fishes. Furthermore, mangrove creek associations were investigated for a commonly occurring marine-spawned estuary-dependent species.

The objectives of the present study were to determine the following:

- The effect of mangrove habitats on the species composition and abundance of juvenile fishes within warm temperate estuaries and the role of habitat, season and physico-chemical factors in determining marine- and estuarine-spawned communities (Chapter 2)
- The role of mangroves within warm temperate estuaries on early stage fish communities as well as the seasonal and physico-chemical variables governing species richness and density (Chapter 3)
- Habitat utilisation and residency of *Rhabdosargus holubi* (Family Sparidae) in two contrasting mangrove habitats using Visible Implant Elastomer tags and stable isotope analysis (Chapter 4)

1.5. THESIS STRUCTURE

This thesis is preceded by a general introduction followed by three related focal chapters and ending with a general synthesis and conclusions. This format has been adopted to facilitate publication of the work and as such there is a limited degree of repetition in the introduction, methods and study areas of each section. The manuscript has been formatted and referenced as per the journal *Estuaries and Coasts*.

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CHAPTER 2

THE EFFECT OF MANGROVE NURSERY HABITATS ON JUVENILE FISH COMMUNITIES IN SELECTED WARM TEMPERATE SOUTH AFRICAN ESTUARIES

2.1. SUMMARY

Tropical mangrove habitats are commonly found to provide critical nursery areas which support a rich and abundant assemblage of fishes by providing shelter from predators and an abundant food supply. The distribution of mangroves however extends into warm temperate regions where studies regarding their relative importance to fish communities are rare but nevertheless assumed to be valuable. Fishes were sampled in neighbouring estuaries with and without mangroves to determine whether this combination of increased refuge and food supply, during critical early life stages, should result in an increased abundance and diversity of juvenile fishes. Results suggested that there is little advantage provided by these habitats within temperate estuaries. Abundance and diversity between estuaries with and without mangroves showed no significant variation, even when fish guilds (marine and estuarine) were treated independently. It is proposed that warm temperate fish fauna, which dominate these systems, have not evolved a dependence on mangroves as the seasonally variable nature of these systems coupled with food availability favours species which are adaptable to their use of the environment.

2.2. INTRODUCTION

Mangroves occur throughout the tropics where they are typically delimited by the 20°C seawater isotherm (Alongi 2009). They do, however, reach further south along the eastern coasts of continents in the southern hemisphere where they extend into warm temperate climatic zones while being represented by few, if not a single mangrove species (Hogarth 2015). Nonetheless, wherever mangroves occur they are a characteristic feature of sheltered intertidal habitats, such as embayment’s and estuaries where the roots form a hard substrate in an otherwise open environment of soft sediment (Hogarth 2015, Nagelkerken et al. 2008). Submerged roots are typically overgrown by algae and epibionts which support numerous aquatic invertebrates and the surrounding detritus-rich muddy substrate forms habitat for various infaunal and epifaunal species (Nagelkerken et al. 2008, Kathiresan and Bingham 2001). Apart from the provision of a suitable and varied habitat, mangrove forests are commonly reported to be among the world's most productive ecosystems and as such contribute a large source of nutrients to surrounding waters via litterfall (Nagelkerken et al. 2008).

This availability of food, shelter and refuge provided by mangroves is commonly cited as a reason for the impressive abundance and diversity of fishes (Nagelkerken et al. 2001) and other aquatic fauna found within these habitats which has resulted in these areas being considered important habitats and notably, as essential fish nurseries (Laegdsgaard and Johnson 2001). Juveniles of many economically important fish species are found to utilise mangrove habitats as a nursery, which implies that mangroves are not only ecologically important but are economically valuable as juveniles later recruit to adult fisheries populations (Baran and Hambrey 1999). Consequently, the global loss of these ecosystems has raised concern (Alongi 2002).

While typically, species richness and abundance of fishes are found to be highest within mangrove habitats (e.g. Gajdzik et al. 2014, Nagelkerken et al. 2001, Robertson and Duke 1987), a number of studies have suggested that neighbouring habitats can also serve an important nursery function. Regardless of the findings, the literature is replete with studies regarding the importance of mangroves as fish nurseries in sub-tropical and tropical regions (Faunce and Serafy 2006) with a definite absence of studies from temperate climatic regions where a different assemblage of fish occurs and mangroves reach their geographical limits. An experimental approach to determine the importance of mangroves as nurseries for juvenile fishes might be to remove all mangroves and study the effects on existing communities (Nagelkerken et al. 2001), which of course is not possible. A suitable indirect method to assess the importance of warm temperate mangroves for juvenile fishes would be to compare different estuaries where mangroves are present or absent but are in the same geographic location and using the same survey method.

Fortunately, as mangroves reach their distributional limits in warm temperate zones, such as along the south-eastern region of South Africa, estuaries with similar geomorphology and hydrology occur alongside one another with only some containing mangroves. The present study therefore aimed to assess the importance of mangroves for juvenile fish communities not by habitat but by using a comparative estuary wide approach where mangrove estuaries would be compared with the nearest neighbouring systems without mangroves. Since it has been widely cited (Verweij et al. 2006, Nagelkerken et al. 2001, Robertson and Duke 1987) that tropical mangroves contribute both to greater species richness and abundance, these response variables were assessed in warm temperate mangroves. It was hypothesised that estuaries where mangroves were present would have both a greater species richness and greater abundance of young fishes when compared to similar estuaries without mangrove habitats.

2.3. MATERIALS AND METHODS

2.3.1. Study area

Sampling was undertaken in the warm-temperate region of south-eastern South Africa (Figure 2.1). A characteristic ichthyofauna of this climatic region is found to occur in estuaries ranging from Cape Agulhas in the west to just south of Port St Johns in the east (Harrison 2002). Four estuaries were selected based on shared similarities which included area of drainage basin, river and estuarine size, mouth state (permanently open) and available habitat (Table 2.1). From the south, the Nahoon Estuary (32° 59'S, 27° 57'E) is situated within the city of East London, it contains the southernmost stand of mangroves in South Africa which were transplanted from Durban Bay and have colonised the lower intertidal areas (Adams et al. 2004). Gonubie Estuary (32° 56'S, 28° 02'E), without mangroves, lies 10 kilometres to the east of the Nahoon. A further 80 kilometres to the north-east, Qora Estuary (32° 27’S, 28° 40’E) is another system without mangroves, while the nearby Xhora Estuary (32 10’S, 29 00’E) is colonised by mangroves throughout the lower regions. Both the Qora and Xhora estuaries fall in the region formerly known as the Transkei which lacks any major development or infrastructure, as such these estuaries are classified by Whitfield (2000) as being in excellent condition with negligible human impact. Conversely, the Nahoon and Gonubie are located in the city area of East London and are popular recreational areas, most notably however is the damning along these river courses which has decreased freshwater inputs to the estuary. Throughout this manuscript, “mangrove presence” refers to the estuary wide presence of mangroves and is not site specific.

Table 2.1: Summary of area cover (ha) of intertidal habitat and total estuarine area per estuary (Adams, unpubl. data).

Estuary	Salt marsh	Submerged macrophytes	Mangroves	Sand/mud banks	Total area
Nahoon	2.8	2.3	1.6	4.5	47.3
Gonubie	3.7	0.8	0	6.3	40.0
Qora	0	8.5	0	10.23	65.2
Xhora	0	2.6	25.5	17.1	91.4

2.3.2. Sampling method

Field sampling took place in January (summer) and July (winter) of 2015 and 2016 at five fixed sampling stations per estuary. Fixed sites were selected remotely for each estuary and were spaced at one kilometre intervals with the first being 500m from the estuary mouth. At each site, physico-chemical measurements were recorded using a YSI-6600 multimeter with probes fitted for temperature (°C), turbidity (NTU), salinity, pH, dissolved oxygen and total dissolved solids (TDS). At each site a 50 x 2m seine net with 12mm stretched mesh was used to sample juvenile fishes. The seine-net was deployed from a boat, covering an area of approximately 400m^2, and was pulled ashore. Care was made to ensure a consistent deployment at all sites and a heavy sinker line allowed the net to be pulled directly through eelgrass beds and pneumatophore habitats to ensure quantifiable results among regions and habitats.

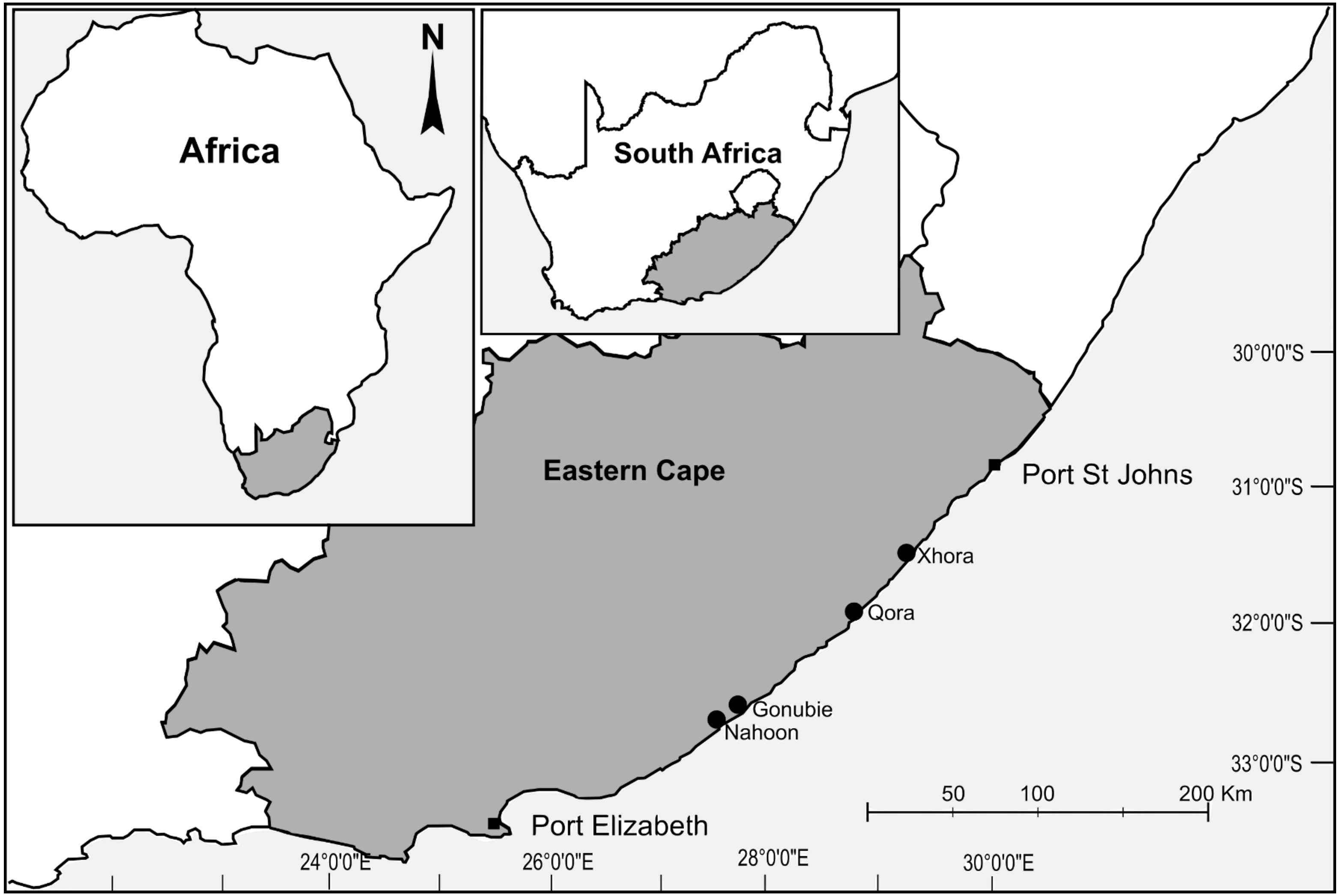

Figure 2.1: Geographic position of study area showing location of study estuaries along the south-eastern warm temperate coast of South Africa.

All fish were identified to species level, measured to the nearest millimetre and quantified before being returned to the water. Those individuals which could not be identified in the field were preserved in 10% formalin for later identification. All fishes were categorised into estuarine usage guilds, as per Potter et al. (2015), with categorisation determined using Whitfield (1998). All species captured fell within either the marine category, species which spawn at sea but show variable dependence on estuaries, or estuarine, species which complete their life cycles within estuaries (Potter et al. 2015).

2.3.3. Statistical analysis

Physico-chemical and biological data were tested for normality and homogeneity of variance prior to statistical analysis. Normality was tested both visually and with the Shapiro-Wilk test. Homogeneity was tested visually by plotting residuals against fitted values and by using Levene's test. Physico-chemical variables did not meet assumptions of normality, even after transformation, therefore non-parametric tests were used. The Kruskal-Wallis test was used to compare each of the environmental variables among estuaries and sites per season due to large seasonal variability. The Mann-Whitney U-test was used to test for differences between seasons and between estuaries with and without mangroves.

Biological data was first assessed for differences between estuaries with and without mangroves with univariate community indices using Student's t-test. Subsequently, marine- and estuarine-spawned guilds were analysed independently as it was anticipated that usage groups would show separate responses to environmental variables. Univariate indices included: number of species (S), representing the total number of species caught at a site, abundance (N) which was Log(x + 1) transformed and represents the catch per unit effort (CPUE) or total number of individuals caught per seine haul, and the Shannon-Weiner diversity index ($H' = -\sum_{I}\,{}_{pi}(\log_{e}\,{}_{pi})$), which represents the number of species and their distribution within a sample. A three-way hierarchical Analysis of Variance (ANOVA) with season and mangrove presence as fixed factors and with estuary as a nested factor was applied to determine variance between and among factors.

Generalised Additive Models (GAMs) were additionally used to quantify the abundance and species richness of the two guilds in relation to environmental factors. GAMs are particularly useful in ecological studies as they can fit a broad range of non-parametric models determined from the observed data and are therefore able to deal with highly non-linear relationships between response and explanatory variables (Guisan et al. 2002). The explanatory variables included those physico-chemical measurements recorded at each site, as well as the factors mangrove presence and site specific habitat (which included substrate type, either sand or mud, and covering vegetation, which was classified as none, eelgrass or mangroves).

Covariates were tested for collinearity by running pairwise plots and examining the variance inflation factors (VIF) of response variables, those exhibiting collinearity were removed from analysis. Variable selection for the models was determined using a forward stepwise approach with evaluation using Akaike Information Criterion (AIC) and by examining the explained deviance of successive models. Mangrove presence was included in all models as it was the factor of primary concern. Untransformed abundance data was modelled using a negative binomial distribution with log link, which was determined as the best fit by visual assessment and AIC score. Species richness was fitted using a Gaussian distribution and log link, which was also determined using visual assessment and comparative AIC scores.

Finally, multivariate analyses were used to determine the relationships among mangrove presence and site specific habitat on fish assemblages. Non-metric multidimensional scaling (nMDS) and analysis of similarity (ANOSIM), based on Bray-Curtis similarity matrix from CPUE, were used to assess whether fish assemblages varied (1) between mangrove presence and (2) among habitats which were based upon sediment type and covering vegetation. The SIMPER routine was used to determine which species were responsible for differences among mangrove presence or habitats. All statistical analyses were performed using R with packages mgcv, VEGAN, and qqplot2 (Oksanen et al. 2007, Wood 2007, Wickham and Chang 2009, RC team 2013). Multivariate analyses were performed using PRIMER v.6.

2.4. RESULTS

2.4.1. Environmental variability

Physico-chemical variability was greater in summer when temperature, salinity and dissolved oxygen showed significant variation among estuaries whereas no significant variability was observed during winter. Temperature was the only factor which differed significantly between summer and winter for all estuaries (*P* < 0.05), while salinity only differed seasonally for the two northern estuaries (Qora and Xhora) where a more pronounced salinity gradient was observed during summer while little freshwater inflow was recorded in winter. Turbidity showed a significant (*P* < 0.05) seasonal difference for the Nahoon and Qora estuaries with dissolved oxygen varying seasonally (*P* < 0.01) for only the Nahoon. Environmental variables measured during the study period are summarised in Table 2.2 and Figure 2.2. Horizontal physico-chemical gradients within estuaries were typically uniform. While gradients were typically more established in summer, particularly temperature and salinity, there was no significant variability among sites within any of the estuaries.

Table 2.2: Range of temperature (°C), salinity, turbidity (NTU) and dissolved oxygen (mg/l) measurements taken at study estuaries for summer and winter of 2015 to 2016.

	Summer			
	Nahoon	Gonubie	Qora	Xhora
Temperature (°C)	17.2 – 24.2	14.2 – 27.4	20.4 – 29.9	19.3 – 30.4
Salinity	29.4 – 35.9	30.6 – 34.8	1.8 – 31.1	12.3 – 35.1
Turbidity (NTU)	0.0 – 18.3	0.0 – 14.4	1.0 – 37.6	0.0 – 11.1
Dissolved oxygen (mg/l)	5.1 – 9.5	7.1 – 8.8	6.9 – 11.1	7.2 – 10.4
	Winter			
Temperature (°C)	15.0 – 18.7	16.0 – 18.8	15.0 – 18.4	15.0 – 18.5
Salinity	31.3 – 35.7	18.9 – 35.8	26.1 – 35.6	30.3 – 35.6
Turbidity (NTU)	0.1 – 5.7	0.1 – 9.2	0.1 – 12.0	0.1 – 18.0
Dissolved oxygen (mg/l)	7.8 – 11.4	6.3 – 10.1	7.6 – 9.0	5.9 – 8.8

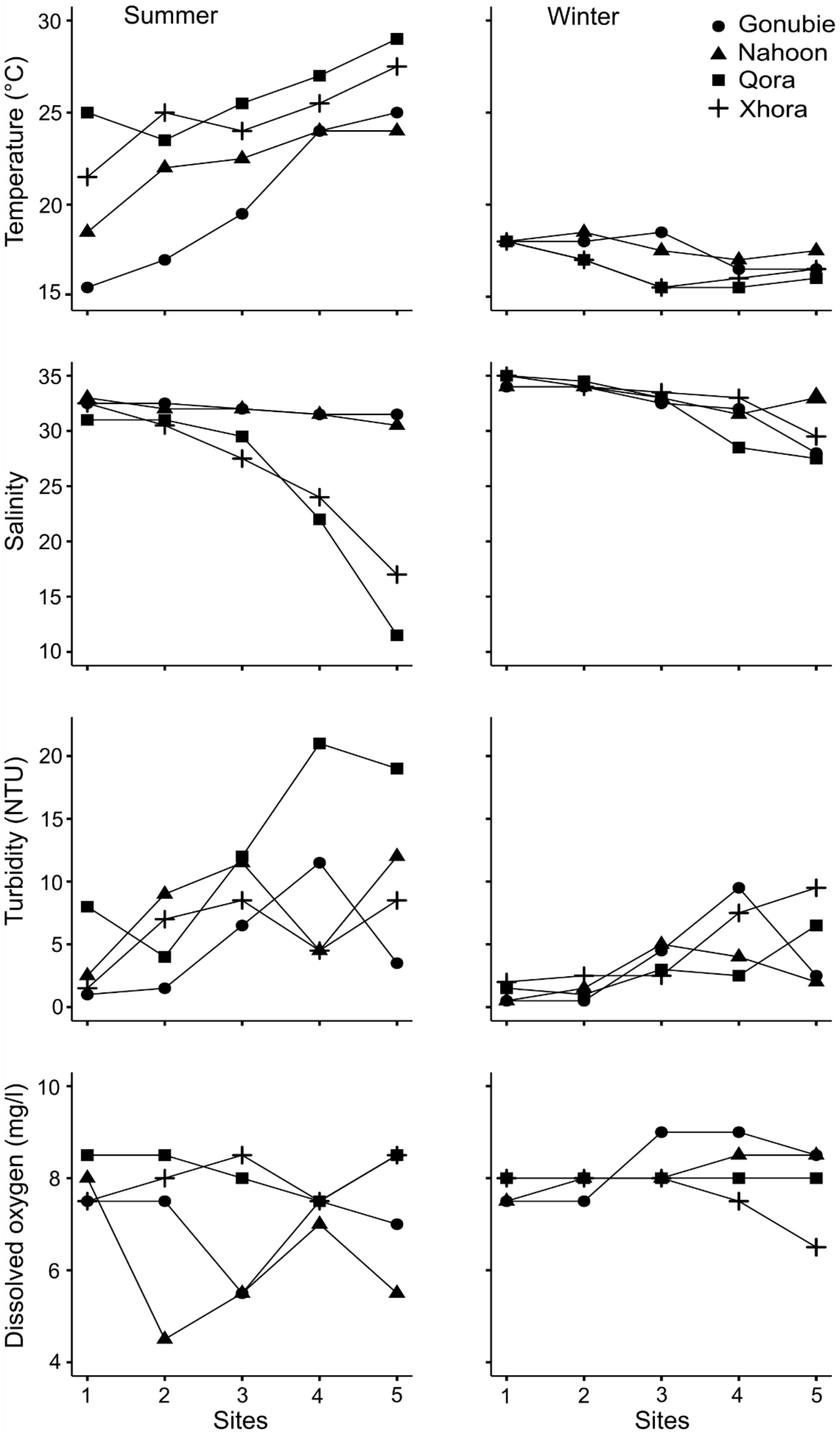

Figure 2.2: Mean temperature (°C), salinity, turbidity (NTU) and dissolved oxygen (mg/l) measurements recorded by site for study estuaries for summer and winter of 2015 to 2016.

Table 2.3: Species composition, catch per unit effort by season, total number individuals captured, percent of total and presence absence of fishes caught from 2015 to 2016.

Guild	Family	Species	Mean CPUE (range)		N	Percent of total	Presence/Absence			
			Summer	Winter			Gon	Nah	Qor	Xho
Estuarine	Ambassidae	*Ambassis dussumieri*	15.03 (0 – 601)	0.68 (0 – 11)	628	4.2		■	■	■
	Atherinidae	*Atherina breviceps*	4.78 (0 – 140)	15.88 (0 – 324)	826	5.5	■	■	■	■
	Clupeidae	*Gilchristella aestuaria*	43.75 (0 – 943)	44.23 (0 – 765)	3519	23.4	■	■	■	■
	Gobiidae	*Caffrogobius gilchristi*	8.15 (0 – 96)	11.90 (0 – 65)	802	5.3	■	■	■	■
		Caffrogobius natalensis	0.43 (0 – 9)	0.30 (0 – 6)	29	0.2	■	■	■	■
		Caffrogobius nudiceps	0.28 (0 – 7)	0.93 (0 – 17)	48	0.3	■	■	■	
		Glossogobius callidus	4.95 (0 – 54)	6.50 (0 – 111)	458	3.0	■	■	■	■
		Glossogobius giurus	0.05 (0 – 2)	0	2	0				■
		Psammogobius knysnaensis	1.15 (0 – 20)	0.63 (0 – 6)	71	0.5	■	■	■	■
		Oligolepis accutipennis	0.13 (0 – 2)	0.18 (0 – 3)	12	0.1			■	■
Marine	Ariidae	*Galeichthys feliceps*	0.88 (0 – 34)	1.33 (0 – 50)	88	0.6	■	■	■	
	Bothidae	*Bothus pantherinus*	0.08 (0 – 2)	0.03 (0 – 1)	4	0		■	■	■
	Carangidae	*Caranx sexfasciatus*	0.43 (0 – 6)	0	17	0.1	■	■	■	■
		Lichia amia	0.13 (0 – 3)	0	5	0	■		■	
	Gerreidae	*Gerres filamentosus*	0.18 (0 – 7)	0	7	0			■	
	Haemulidae	*Pomadasys commersonnii*	9.72 (0 – 74)	2.05 (0 – 17)	471	3.1	■	■	■	■
		Pomadasys olivaceum	0.65 (0 – 21)	1.50 (0 – 13)	86	0.6	■	■	■	■
	Leiognathidae	*Leiognathus equulus*	0.03 (0 – 1)	0	1	0			■	
	Lutjanidae	*Lutjanus fulviflamma*	0	0.03 (0 – 1)	3	0				■
	Monodactylidae	*Monodactylus falciformis*	0.5 (0 – 15)	1.40 (0 – 35)	76	0.5	■	■	■	■
		Monodactylus argenteus	0.08 (0 – 2)	0	3	0				■
	Mugilidae	*Myxus capensis*	2.33 (0 – 37)	7.55 (0 – 107)	395	2.6	■	■	■	■
		Mugil cephalus	0.03 (0 – 1)	0.35 (0 – 8)	15	0.1	■	■	■	■
		Liza richardsonii	3.75 (0 – 27)	14.78 (0 – 469)	741	4.9	■	■	■	■

	Liza tricuspidens	1.42 (0 – 14)	4.80 (0 – 139)	249	1.7	■	■	■	■
	Liza dumerilii	1.65 (0 – 43)	2.20 (0 – 53)	154	1.0	■	■	■	■
	Liza macrolepis	0.15 (0 – 43)	0.68 (0 – 11)	33	0.2	■	■	■	■
Platycephalidae	*Platycephalus indicus*	0	0.10 (0 – 2)	2	0			■	■
Rhinobatidae	*Rhinobatos annulatus*	0.02 (0 – 1)	0	1	0	■			
Sciaenidae	*Argyrosomus japonicas*	0.30 (0 – 5)	0.03 (0 – 1)	13	0.1		■	■	■
Siganidae	*Siganus sutor*	0.05 (0 – 2)	0	2	0		■		
Solidae	*Heteromycteris capensis*	1.07 (0 – 23)	1.35 0 – 16)	97	0.6	■	■		
	Solea turbynei	4.22 (0 – 24)	5.20 (0 – 33)	377	2.5	■	■	■	■
Sparidae	*Acanthopagrus vagus*	0.05 (0 – 1)	0.05 (0 – 2)	4	0			■	■
	Rhabdosargus holubi	78.87 (0 – 487)	62.43 (0 – 379)	5652	37.5	■	■	■	■
	Diplodus capensis	1.55 (0 – 17)	0.10 (0 – 4)	66	0.4	■	■		
	Diplodus cervinus	0.03 (0 – 1)	0	1	0		■		
	Lithognathus lithognathus	0.08 (0 – 2)	0	3	0	■	■		
Sphyraenidae	*Sphyreana jello*	0.05 (0 – 2)	0	2	0				■
Terapontidae	*Terapon jarbua*	0.15 (0 – 3)	0.80 (0 – 22)	38	0.3	■	■	■	■
Tetraodontidae	*Amblyrhynchotes honckenii*	0.90 (0 – 21)	0.25 (0 – 4)	46	0.3	■	■	■	■
Torpedinidae	*Torpedo sinuspercisi*	0.05 (0 – 1)	0.15 (0 – 2)	8	0.1	■	■		■

2.4.2. Species composition and guild

A total of 15 062 fishes were caught from the four estuaries comprising 44 different taxa representing 23 families (Table 2.3). The most speciose family was Gobiidae with seven taxa, followed by Mugilidae and Sparidae with six and five species recorded, respectively. Marine-spawned fishes accounted for nearly 60% of the total catch and were represented by 34 fish taxa compared to estuarine-spawned with 10 species. The single most abundant species, accounting for 38% of the total catch, was *Rhabdosargus holubi* with 5652 individuals captured. The second most abundant species was *Gilchristella aestuaria* with 3519 individuals captured. Eight species accounted for nearly 90% of the total abundance with the majority of the remaining species typically being represented by fewer than 50 individuals (Table 2.3). Marine species were marginally more abundant in summer but species richness did not vary between summer and winter. Estuarine species abundance was higher in winter while also being represented by, on average, more species.

2.4.3. Fish communities and estuary type

The univariate indices representing number of species present (S), abundance (N) and Shannon’s diversity (H’) all showed no significance in variation when compared between estuaries with and without mangroves (Table 2.4). This finding was consistent when indices were compared seasonally.

Species were categorised into estuarine and marine for further analysis on the effects of mangroves on communities. Average values of the Shannon’s diversity index and mean species richness for marine-spawned species showed that averages were typically higher in the Gonubie and Nahoon (Figure 2.3) where a lower salinity gradient was typically evident. Shannon’s index for the Qora Estuary had the lowest mean for marine species while still having a comparable species richness indicating dominance by relatively few species. This dominance (as for the other estuaries in this study) was due, primarily, to the high abundance of *R. holubi* at all sites as well as the absence of any other marine species at the uppermost site in the Qora Estuary. Mean CPUE for marine and estuarine species was consistently high at Qora. Apart from *R. holubi,* other species such as *P. commersonnii*, *L. richardsonii* and *G. aestuaria* occurred in the Qora Estuary in particularly high abundances. Results from a three-way analysis of variance showed no difference between estuarine mangrove presence (or estuary) for both estuarine- and marine-spawned categories (Table 2.5). Species richness and Shannon's index did however vary between seasons for the estuarine-spawned group ($P < 0.01$).

Table 2.4: Results from Students *t-test* comparing selected univariate indices of fish assemblages from estuaries with and without mangroves.

	Two Sample t-test		
Index	*t-stat*	*df*	*P*
No. species (S)	0.53	77.82	0.60
Log(x+1) (N)	1.03	69.47	0.30
Shannon's (H')	0.54	77.75	0.59

Table 2.5: F-ratios and significance levels from three-way hierarchical ANOVA of selected univariate indices of estuarine and marine fish assemblage guilds, Estuary nested within Mangrove.

		Estuarine			Marine	
	Mangrove	(Estuary)	Season	Mangrove	(Estuary)	Season
No. species (S)	0.08 ns	0.56 ns	10.93 **	0.87 ns	1.07 ns	0.33 ns
Log(x+1) (N)	0.01 ns	0.71 ns	1.66 ns	1.90 ns	0.62 ns	0.28 ns
Shannon's (H')	0.01 ns	0.07 ns	7.22 **	0.01 ns	0.09 ns	0.08 ns

*(significance codes ***P <0.001; ** <0.01; * <0.05; ns non-significant)*

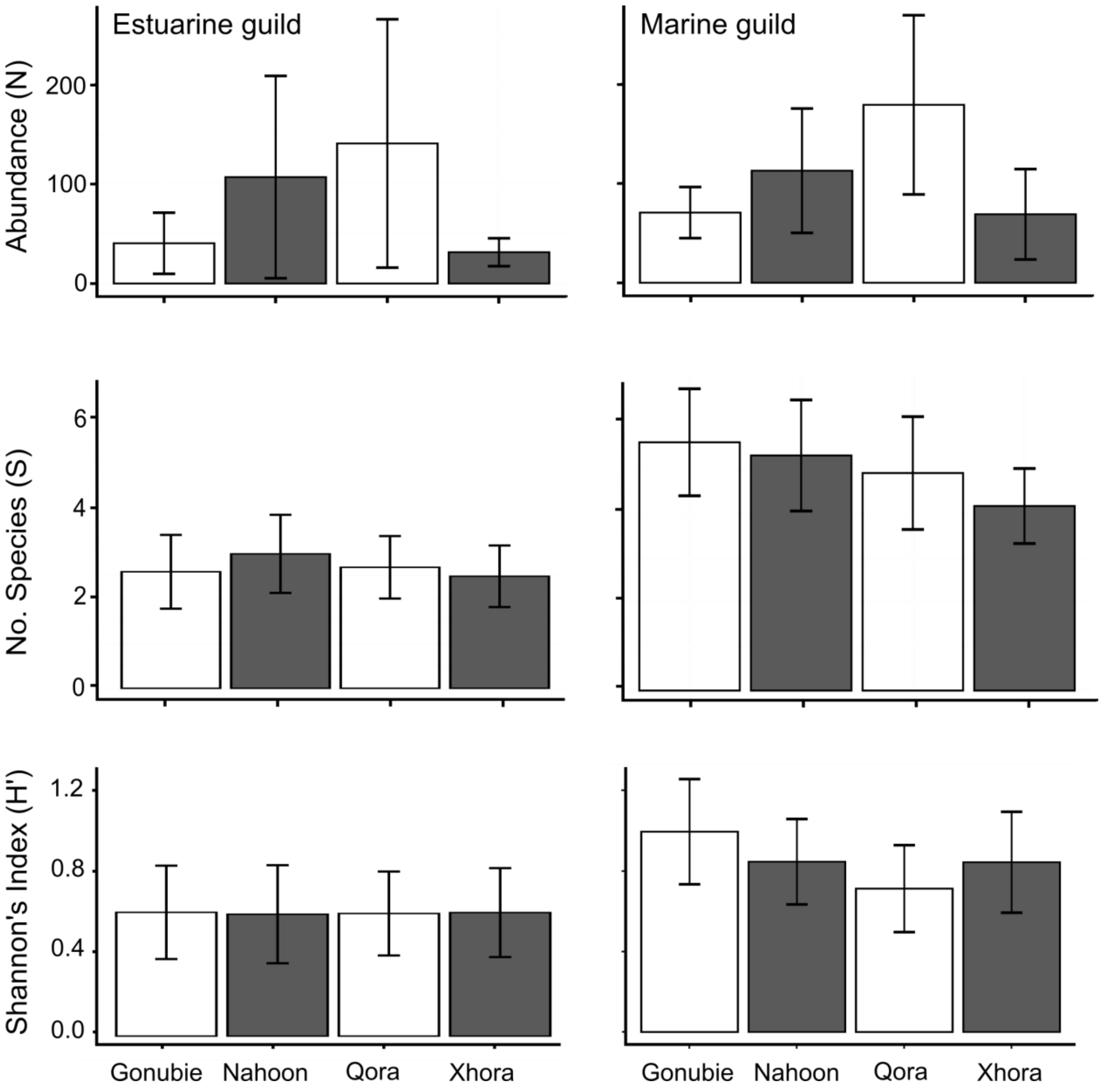

Figure 2.3: Mean (± 95% confidence intervals) CPUE, species richness and Shannon's diversity index for estuarine- and marine-spawned fishes per estuary (open bars = mangrove absent, shaded bars = mangrove present).

2.4.4. Spatial distribution and environmental factors

Generalised Additive Models were used to further test for factors responsible for the observed distributions of fishes. The presence or absence of mangroves within an estuary was included as a factor in all models as this was of primary concern. Two response variables, species richness and abundance, were analysed for each of the guilds (Estuarine and Marine), results are presented in Table 2.6 and Figure 2.4.

Abundance of estuarine fishes was best described by a model including temperature and turbidity. Abundance peaked at temperatures between 19 and 25°C and at turbidity's ranging between 3 and 10 NTU. Richness of estuarine species similarly appeared to not be influenced by the presence of mangroves but rather was sediment specific, sandy sediments were found to have less species than muddy sediment regardless of covering vegetation being present or not. Additionally, temperature and turbidity were associated with changes in species number. Temperature was particularly influential ($P < 0.001$) where species richness increased with declining water temperatures and salinities and increased at higher turbidity's.

Variations in abundance for marine-spawned fishes were best described by a model including temperature and dissolved oxygen. Presence of mangroves was found to have no influence on abundance trends. Like that of estuarine-spawned, abundance peaked at temperatures between 20 and 25°C and showed a bimodal trend for dissolved oxygen with peaks at intermediate (6 mg/l) and high values (9 mg/l). Species richness was influenced both by salinity and turbidity with no response to presence of mangroves or habitat types. The number of marine-spawned species present declined with decreasing salinity and showed a strong relationship with turbidity where clear water, below 5 NTU, was characterised by very low catches.

Table 2.6: Results of Generalised Additive Modelling with mangrove as fixed factor and predictive variables selected by forward stepwise approach for abundance and richness of estuarine- and marine-spawned fishes.

Category	Factor	Z value (factor)	Chi.sq (smoother)	Deviance explained (%)
Estuarine – abundance*	Mangrove presence/absence	0.18 ns		
	Temperature		7.33 *	
	Turbidity		21.26 ***	
	Dissolved 02		16.48 ***	36.30
Marine – abundance	Mangrove presence/absence	1.66 ns		
	Temperature		7.78 *	
	Dissolved O2		18.63 ***	20.60
		T-value (Factor)	F (smoother)	
Estuarine – richness*	Mangrove presence/absence	0.38 ns		
	Habitat (sand)	-3.17 **		
	Temperature		2.69 ***	
	Salinity		0.70*	
	Turbidity		1.16 *	55.70
Marine – richness	Mangrove presence/absence	1.62 ns		
	Salinity		0.78 *	
	Turbidity		3.12 **	42.70

**Abundance fitted with negative binomial distribution*

**Richness fitted with Gaussian distribution (significance codes ***P <0.001; ** <0.01; * <0.05; ns = non-significant)*

2.4.5. Multivariate analysis

Analysis of Similarity (ANOSIM) and nMDS results showed no difference in fish assemblages for either estuarine- or marine-spawned categories between estuaries with and without mangroves. Fish assemblages (both categories) however did show some variation among habitats (Table 2.7 and Figure 2.5). Site specific sediment type appeared to be the primary factor separating assemblages with presence or absence and type of vegetation acting as a secondary factor. Pairwise tests revealed that differences between sand and mud resulted in high variability (ANOSIM R-Statistic > 0.5, P < 0.001). Differences in fish assemblages between mangroves and *Zostera* over muddy sediments, however, were very low (R-Statistic < 0.1) indicating usage of these areas by similar groups.

Sediment type differences affecting assemblage structure were shown by catch differences of estuarine-spawned *Caffrogobius gilchristi* (22%), *Atherina breviceps* (19%), and *Gilchristella aestuaria* (18%). While marine-spawned *Rhabdosargus holubi* (22%), *Solea turbynei* (16%), *Liza richardsonii* (9%) and *Pomadasys commersonnii* (8%) contributed to differences between assemblage structure of varying sediment type.

Table 2.7: Results from Analysis of Similarity for estuary type, estuary and habitat type per estuarine use category (P = significance level of sample statistic).

Factor	Estuarine		Marine	
	Global R	P	Global R	P
Mangrove presence/absence	0.011	0.702	0.019	0.096
Estuary	0.039	0.055	0.078	**0.001**
Habitats	0.332	**0.001**	0.158	**0.001**

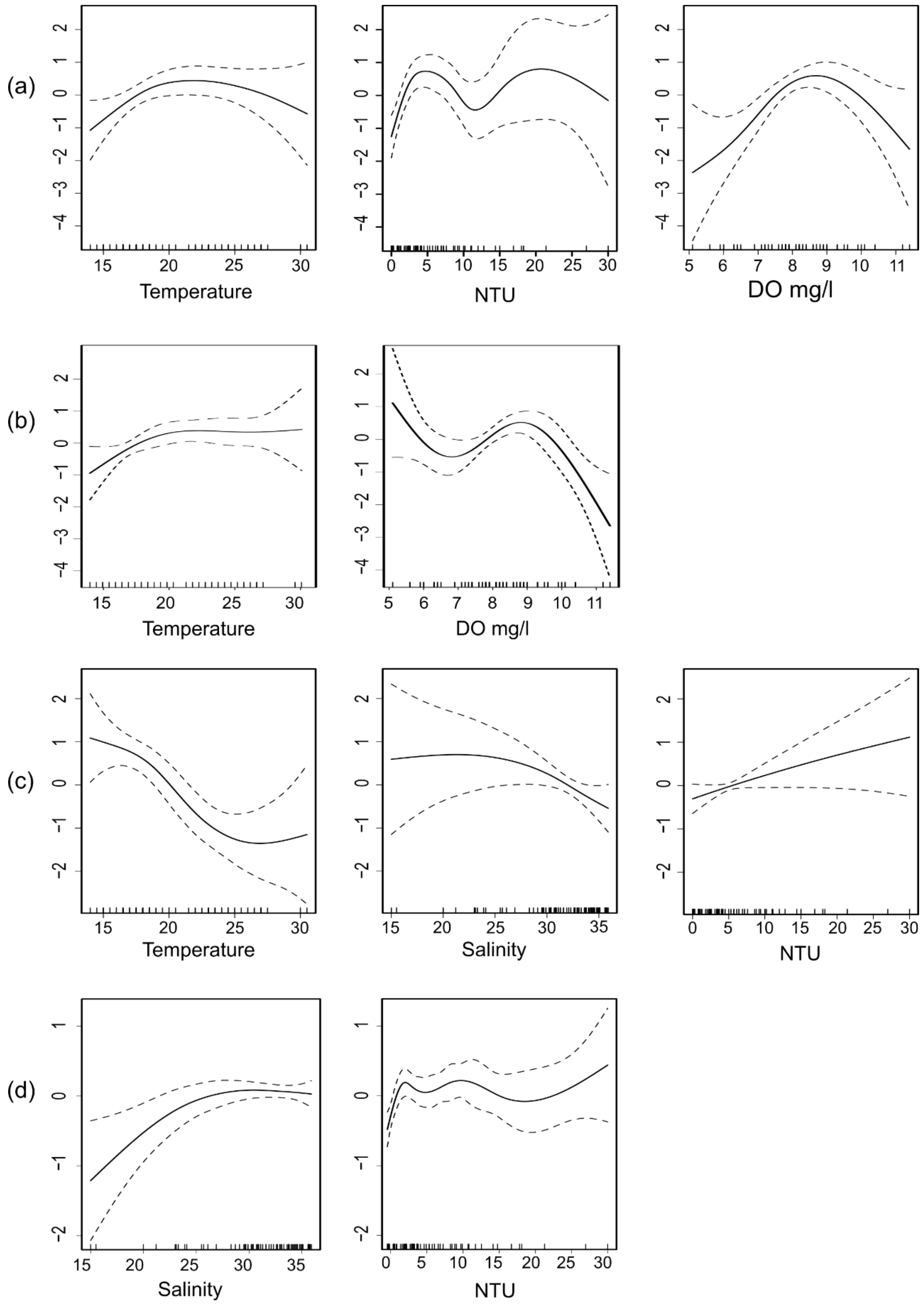

Figure 2.4: Outputs from GAM's illustrating relationship between response variables (abundance and species richness) with environmental predictors, rows include (a) estuarine – abundance, (b) marine – abundance, (c) estuarine – richness, and (d) marine – richness and correspond with results in table 2.6 for fishes collected from study estuaries during summer and winter 2015 – 2016.

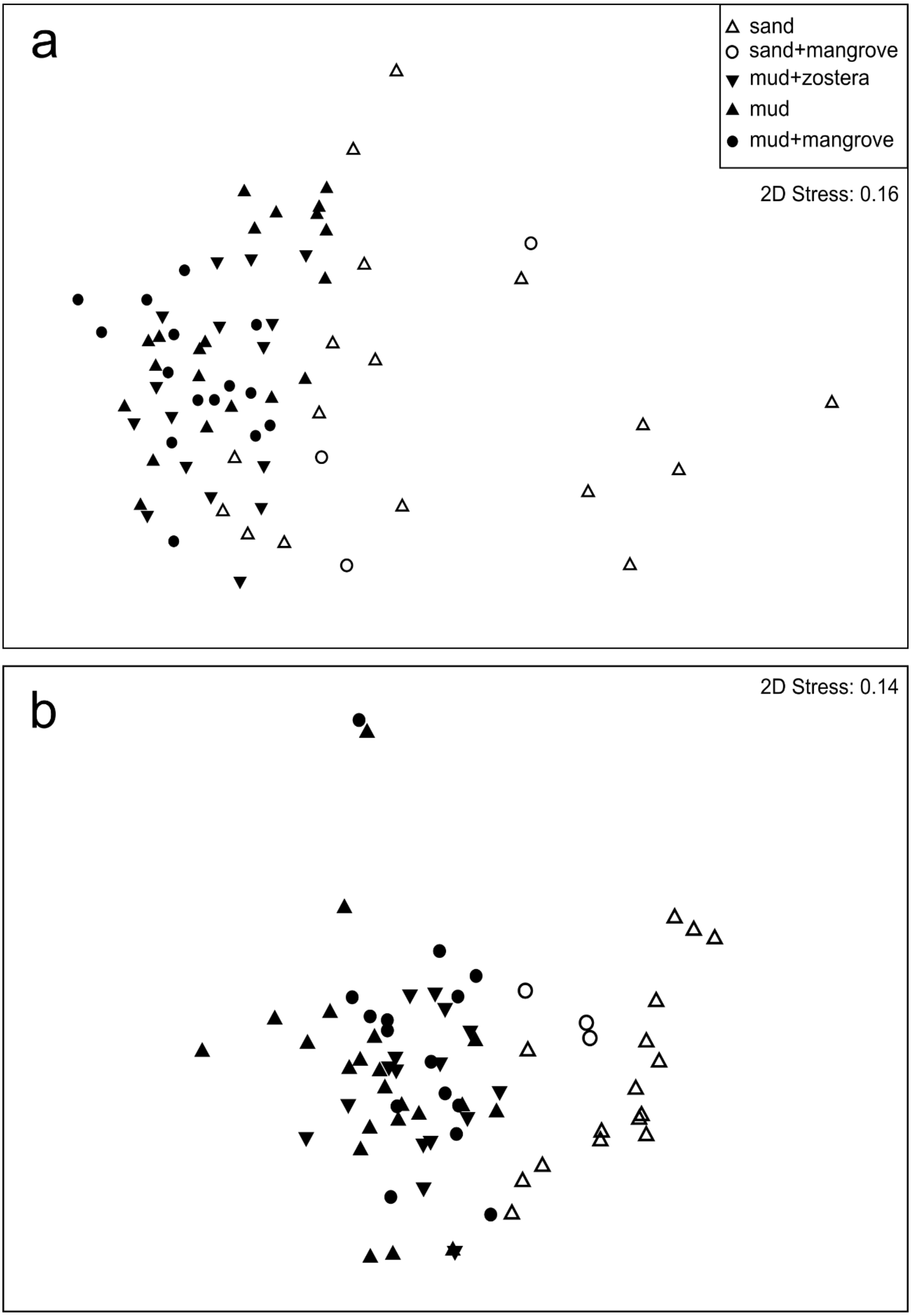

Figure 2.5: Non-metric Multidimensional Scaling results for species composition by habitat type for (a) marine- and (b) estuarine-spawned guilds of juvenile fishes captured from study estuaries over summer and winter 2015-2016.

2.5. DISCUSSION

This study aimed to evaluate the importance of mangroves, as an added aquatic habitat, for fish communities in temperate South African estuaries. This has not been previously assessed in South Africa and it was unknown whether temperate mangroves are as important to fishes as their tropical counterparts. Variation in fish communities among estuaries with and without mangroves was low and showed high similarity of both estuarine- and marine-spawned guilds. Sampled fish communities were representative of a South African warm temperate ichthyofauna with high average similarity among estuaries, made evident by a low ANOSIM *R* statistic (near zero) (Clarke and Warwick 1994). Generalised Additive Models similarly showed that communities were occupied by a warm temperate fish assemblage by the observed response to temperature. Abundance for both estuarine- and marine-spawned fishes was highest at intermediate temperatures (~22°C) after which it declined, while species richness of the two guilds peaked at temperatures below 20°C. The study found that the presence of mangroves within estuaries had no significant effect on juvenile fish communities among the sampled estuaries. Neither of the predicted effects of increased species richness or increased abundance was observed for estuaries where mangroves were present.

Beck et al. (2001) suggested that four biotic factors contribute largely to the success of a fish nursery area, these include the supply of larvae/juveniles to stock these habitats, limited interspecific competition, abundant and suitable food supplies, and a moderate degree of shelter from predation, particularly during the early stages. The latter two factors, food supply and shelter, are frequently suggested as major features offered by mangrove habitats resulting in their characteristic association with a diverse and abundant fish community (Nagelkerken et al. 2008, Faunce and Serafy 2006). Indeed, experimental testing within the field and lab under tropical conditions have shown that densities of juvenile fish increase in artificial mangrove structure (Nagelkerken et al. 2010) and particularly when artificial structure has accumulated algae and other epiphytic growth (Verweij et al. 2006, Laegdsgaard and Johnson 2001). Additionally, the presence of predators leads to smaller juveniles actively seeking shelter, while larger juveniles, perhaps combined with ontogenetic diet changes and decreased vulnerability with size, become less attached to these habitats (Laegdsgaard and Johnson 2001).

Typically, most studies have found that within estuaries or embayment's, fish assemblages are more diverse and/or individuals are more abundant within mangrove habitats when compared with alternative, often open habitats (e.g. Nanjo et al. 2014, Nagelkerken et al. 2008, Robertson and Duke 1987). Whereas studies by Huxham et al. (2004), Payne and Gillanders (2009) and Wang et al. (2009) found no difference between, or indeed higher densities within open non-mangrove habitats. The majority of these studies have however compared nearby habitats, often within tens of meters, potentially neglecting the greater impact of mangroves and the variable movement patterns of fish (such as diurnal or feeding migrations). A true determination of the impact of a feature, such as mangroves with its added refuge and/or feeding potential, may well be operating at a larger scale than that of its immediate habitat (Nagelkerken et al. 2015, Sheaves et al. 2015). For example, Nagelkerken et al. (2001), studied juvenile fish assemblages of eleven Caribbean inland bays with varying occurrence of mangroves and seagrass. Fish communities of mudflats, seagrass beds and mangroves were similar in bays where mangroves were present and were characterised by an abundant assemblage of species which utilise shallow waters as nurseries. Conversely, mudflats and even seagrass beds of bays where mangroves were not present had depauperate fish assemblages with lower average abundances and often lacked shallow-water nursery species. This disparity was attributed by the authors to the variable movement of species or individuals amongst habitats and concluded that the combination of mangroves and seagrass enhance densities and richness on nearby mudflats or other adjacent habitats (Nagelkerken et al. 2001). Conversely, the current study found that warm temperate South African juvenile fish communities associated with mudflats, eelgrass and (when present) mangrove habitats were largely similar, regardless if they were from estuaries with or without mangroves.

The similarity of fish communities among study sites may be attributed to a variety of factors. The functional role of mangroves in refuge provision, for example, may be quite like that offered by other habitats typically found in the estuarine environment (Nagelkerken et al. 2008). The shallow littoral waters of estuaries are found to provide a refuge for many small sized or juvenile fishes (Strydom 2015) and the disappearance of aquatic vegetation from an estuary has been linked to changes in distributions of small fishes using the shallows as an alternative refuge habitat (Ruiz et al. 1993). Eelgrass beds similarly provide a valuable nursery habitat and Weerts and Cyrus (2002) found comparatively higher abundances and a greater diversity of fishes in eelgrass beds when compared to neighbouring mangrove habitats and mudflats in subtropical South Africa. Additionally, temperate mangrove ecosystems are found to be significantly less productive than their tropical counterparts with lower litter production as a source of input for organic matter and nutrients (Bouillon et al. 2008). Subsequently, little variation has been observed in the abundance and biomass of meiofauna between temperate mangrove sediments and mudflats as well as sediments from estuaries without mangroves (Morrisey et al. 2010, Chapman and Tolhurst 2004). Likewise, epifaunal densities of pneumatophores were found to be measurably lower than those found on eelgrass blades or saltmarsh grasses (Gwyther 2002). The relative contribution of temperate mangrove habitats to fish production may therefore be quite limited as both refuge and food availability may not be greater within these habitats.

Though typically, more diverse systems result in more diverse fish assemblages (Kovalenko et al. 2011, Whitfield 1999), estuaries, and particularly those in South Africa, may provide sufficient habitat variability and refuge through other factors such as water turbidity and sharp environmental gradients which may otherwise act as predator deterrents, as well as protection from a dynamic and turbulent nearshore (Whitifield and Pattrick 2015). Additionally, fishes may show adaptive habitat responses and either restrict movement

(Carrasou et al. 2016) or exhibit plasticity in use in estuaries (Edworthy and Strydom 2016). Though referring to nematodes, Hodda (1990) argued that the stochastic variation of environmental and habitat factors in temperate mangroves favours adaptability rather than specialisation, so too can this be said of warm temperate estuarine ichthyofauna. While tropical mangroves are typically occupied by a characteristic assemblage of fishes, which may benefit from greater refuge and feeding opportunities in these productive and stable habitats, warm temperate mangrove counterparts appear to provide a region where the abundance of food items is no greater than alternate habitats.

The adaptability of temperate estuarine ichthyofauna is exemplified by the low variance of fish communities among habitat types. Apart from communities either being associated with sand or mud sediments, there was little difference amongst those communities found in mangrove, eelgrass or non-vegetated sites. Seasonal variation is a characteristic feature of temperate systems and this is particularly evident in estuaries where temperature and rainfall are strongly associated with the biomass of important food items including plankton (Froneman 2001) and meiofauna (Castel et al. 1989). The absence of any mangrove or eelgrass associated fish species may be indicative of this seasonality where the most evolutionary stable strategy is for fish species to be adaptable in their use of habitats where refuge and food availability show strong seasonal variation.

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CHAPTER 3

THE EFFECT OF MANGROVE PRESENCE ON EARLY STAGE FISH COMMUNITIES IN SELECTED WARM TEMPERATE SOUTH AFRICAN ESTUARIES

3.1. SUMMARY

Assemblages of early stage fishes (larval and early juvenile) were investigated and compared among four estuaries where mangroves were either present or absent, in the temperate biogeographic region of South Africa. Early stage fishes were collected by means of boat based plankton tows in summer and winter over two years in 2015 and 2016. A total of 12 597 early stage fishes were collected representing 24 families and 47 taxa. Estuarine-spawned fishes dominated catches with three species, *Gilchristella aestuaria, Caffrogobius gilchristi* and *Glossogobius callidus*, accounting for nearly 80 percent of the total catch. Density, species richness and diversity of early stage fishes did not differ between estuaries with or without mangroves. Densities of both estuarine- and marine-spawned species were found to be significantly influenced by freshwater input while species richness positively related to freshwater input and latitude despite catches still representing typical warm temperate fish communities. Whereas temperate estuaries undeniably serve as nurseries for a range of species, their temporal stochasticity and seasonal variability has resulted in generalist species able to capitalise on a range of habitats with few specialists. Natural freshwater input does however remain a critical component for the high productivity and nursery value of these systems.

3.2. INTRODUCTION

Shallow water seascapes, such as lagoons, embayments or estuaries provide key nursery areas in sustaining ecologically and economically important species (Beck et al. 2001). Research has largely focussed on the differential nursery value of specific habitats on recipient populations within these seascapes (Dahlgren et al. 2006, Beck et al. 2001).

However, studies which have assessed nursery environments rather than habitats have noted the contribution of a variety of habitat types on the fish communities with more diverse systems containing both greater diversity and greater abundance of species (Messmer et al. 2011; Mumby et al. 2004; Nagelkerken et al. 2001).

The process of valuing nursery habitats and their importance to fishes has identified that many assessments have taken a static approach by considering habitats as isolated entities. Contemporary research on motile species should consider the complexity and dynamics of coastal ecosystems exemplified by the ‘seascape nursery’ concept developed by Nagelkerken et al. (2015) and Sheaves et al. (2015). The concept defines a nursery as a spatially explicit seascape consisting of a mosaic of habitats which are functionally connected thereby incorporating ecological habitat linkages by motile species (Nagelkerken et al. 2015). Strong connectivity among habitat patches, which includes settlement areas, shelter and feeding sites, plays a role in the population dynamics and ultimately stock replenishment of nurseries (Nagelkerken et al. 2015).

Nurseries vary in their value to recipient fish populations depending on the provision of suitable physiological conditions, availability of suitable prey and provision of refuge offered by habitat heterogeneity (Sheaves et al. 2015). Among these, the value of mangrove forests as nurseries and intermediary habitats for tropical fish assemblages has been especially noted where densities and biomass of fishes in adjacent and adult habitats are often greater resulting in greater larval supply to receiving environments (Nagelkerken et al. 2012; Mumby et al. 2004). This added value is often attributed to two main features provided by mangroves: increased refuge from predators due to a structurally heterogenous environment and the provision of a diverse and abundant food source (Nagelkerken et al. 2008; Robertson and Blaber 1993).

Studies investigating the importance of mangroves to early stage fish communities in temperate climates are rare and altogether absent in South Africa. Larval fish studies in estuaries have typically been confined to single systems where assemblages are assessed in response to environmental gradients and seasonality (Wasserman et al. 2010; Pattrick et al. 2007); while multi-system studies have compared fish assemblages between climatic zones or estuary types (Strydom 2015; Whitfield 1994). Alternately, larval or juvenile fish community studies have taken place within systems where communities are compared using a habitat by habitat approach (Weerts and Cyrus 2002; Able 1999).

As larval fish communities reflect both the presence and biomass of adult stocks (Mangel and Smith 1990), sampling of early stage fishes should reflect whether mangroves either contribute to greater survival and ultimately biomass and/or unique communities of reproductive adults. The aim of this study was therefore to compare the species composition, estuarine association, species richness and abundance of early stage fishes between two groups of, otherwise similar, warm temperate South African estuaries where mangroves were present in one group and absent from the other. Furthermore, this study aimed to investigate the physical, temporal and spatial factors governing assemblages as well as the species composition of early stage fishes in an area of South Africa where no ichthyofaunal studies have taken place.

3.3. MATERIALS AND METHODS

3.3.1. Study area

Early stage fishes were collected from four estuaries along the south-eastern coast of South Africa (Figure 3.1). Estuaries were selected based on shared similarities in mouth state (permanently open), drainage area, estuary and river length, estuary area, as well as shared habitat availability (barring mangroves) (Table 3.1). Estuaries were grouped into those with mangroves (Nahoon and Xhora) and those without (Gonubie and Qora). Mangroves in the Nahoon Estuary represent the southernmost stand in South Africa at nearly 33°S, they are not naturally occurring however having been transplanted from Durban harbour in 1969 but have nevertheless colonised the lower reaches of the estuary (Adams et al. 2004). Mangroves of the Nahoon are represented by a single species, *Avicennia marina*.

Mangroves of the Xhora Estuary are naturally occurring, three species are present which include *Rhizophora mucronata, Bruguiera gymnorrhiza* and *A. marina,* the latter being the most abundant. The ichthyofauna of the study region is characterised by a warm temperate assemblage which is found to occur from Cape Agulhas at the south western tip of Africa to just south of Port St. Johns in the east (Harrison 2002), approximately 80 km north of Xhora Estuary mouth.

Table 3.1: Physical characteristics and area (ha) coverage of sampled estuaries on the temperate south east coast of South Africa (Adams, unpubl data, Harrison 2002).

Estuary	Coordinates at mouth	Drainage area	Total estuarine area	Average depth (m)	Mangrove area
Nahoon	32°59’S; 27°57’E	54 800	47.30	2.32	1.60
Gonubie	32°56’S; 28°02’E	66 500	40.00	1.68	0
Qora	32°27’S; 28°40’E	70 000	65.20	1.03	0
Xhora	32°10’S; 29°00’E	43 800	91.40	2.10	25.50

3.3.2. Field sampling and laboratory techniques

Sampling of early stage fishes took place biannually over summer and winter of 2015 – 2016. All sampling took place at night, beginning approximately 30 minutes after sunset. Samples were collected using two WP2 plankton nets (570 mm mouth diameter with 0.2 mm mesh aperture size) fitted with Kahlsico 005 WA 130 flow meters. Nets were simultaneously deployed from either side of the bow of a motorised boat. Two subsurface samples (replicates) were collected at each of 5 sites, spaced 1 km apart in each estuary. Nets were maintained in the upper 0.8 m of the water column and were towed at a speed of approximately 1 to 2 knots for 3 minutes. The average water volume filtered by nets was 18.9 m^3 (S.D. ± 6.29). Samples were preserved on site with 10% buffered formaldehyde for later processing in the laboratory with sorting taking place under a stereo dissection microscope to remove all larval and early juvenile fishes. Fishes were identified to the lowest possible taxon according to Smith et al. (2003), Leis and Carson-Ewart (2000) and Neira et al. (1998)

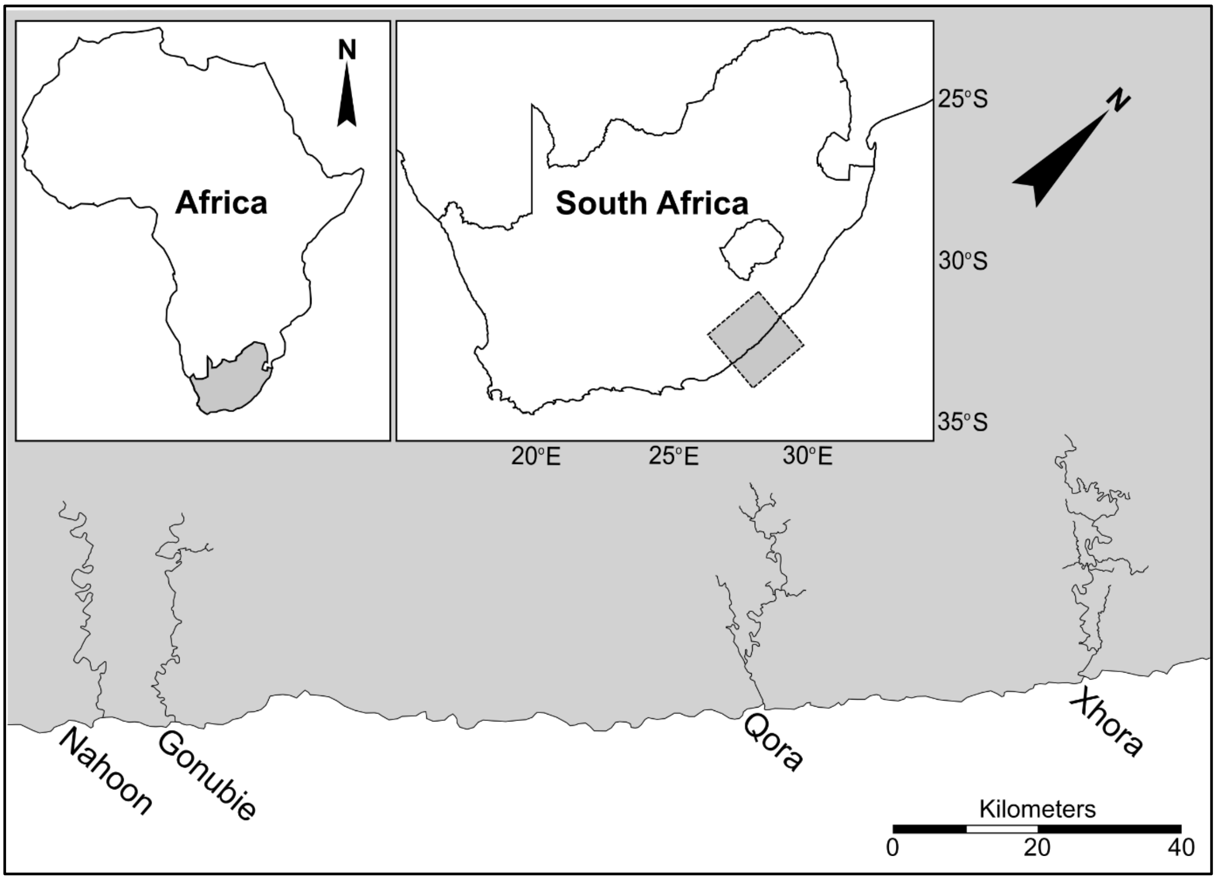

Figure 3.1: Geographic position of study estuaries along the temperate south-eastern coast of South Africa.

All identified early stage fishes were separated into estuarine usage categories, as per Potter et al. (2015), with categorisation determined using Whitfield (1998) and Smith et al. (2003). All species identified were either in the marine category, those species which spawn at sea but show variable dependence on estuaries, or in the estuarine category, those species which complete their entire life cycles within estuaries (Potter et al. 2015) and are referred to as estuarine-spawned and marine-spawned, respectively, throughout the manuscript. The term ‘early stage fishes’ is used to collectively designate all larval, transforming (settlement) and early juvenile stages. Fishes were measured to the nearest 0.1 mm body length (BL), which represents notochord length in preflexion and flexion larvae, and standard length in postflexion larvae and early juveniles. Measurements were made using an eyepiece micrometer for all larvae and Vernier calipers for larger specimens. Density of early stage fishes is expressed as number of individuals per 100 m^3 of water. Density was calculated using a formula based on a predetermined calibration value for each flow meter used:

$$\text{Density} = \frac{\text{Number of fish per haul}}{(\text{Flow meter revolutions / calibration value in m}^3)} \times 100$$

Environmental measurements were recorded directly following a sampling event and taken at 0.5 m intervals between the surface and bottom of the water column at each site in each estuary using a YSI 6600 multiparameter meter. Measurements included temperature (°C), salinity, water turbidity (NTU), total dissolved solids (TDS), pH and dissolved oxygen (mg/l).

3.3.3 Data analyses

Environmental and biological data were tested for normality and homogeneity of variance both visually and using Shapiro-Wilk and Levene’s test, respectively. Environmental variables were assessed using the Kruskal-Wallis rank sum test as assumptions for parametric tests were not met. Univariate metrics of biological data (density, Shannon’s index and species richness) were assessed using one-way analysis of variance (ANOVA) after being log-transformed to meet parametric assumptions. Environmental and biological data was assessed between estuaries, seasons, sites and mangrove presence/absence. Replicate values of biological data were used per site, i.e. no data were averaged prior to statistical tests.

Generalised Additive Models (GAMs) were used to further investigate the relationships between fish density and richness with environmental variables and mangrove presence or absence. These analyses were conducted using density of all fishes captured, estuarine- and marine-spawned separately, as well as individual density of the most commonly occurring species. GAMs were utilised as they are semiparametric extensions of generalised linear models that are effective for describing non-linear relationships between predictor and response variables, which are common in environmental studies (Guisan et al. 2002). Explanatory variables included those environmental variables recorded at each site as well as the factor estuarine mangrove presence or absence. Data exploration was performed following Zuur et al. (2010) to avoid statistical problems commonly encountered when performing GAMs.

Covariates were tested for collinearity by running pairwise plots and examining the variance inflation factors (VIF) of response variables, those variables exhibiting collinearity were removed from analysis or analysed separately. Variable selection for the models was determined using a forward stepwise approach with evaluation using Akaike Information Criterion (AIC) and by examining the explained deviance of successive models. Mangrove presence was included in all models as it was the factor of primary concern. Untransformed density data were modelled using a negative binomial distribution with log link, which was determined as the best fit by visual assessment and AIC score. Species richness was fitted using an exponential distribution with log link, which was also determined using visual assessment and comparative AIC scores.

Multivariate analyses were performed on fish communities to determine relationships between individual estuaries, mangrove presence/absence and season using PRIMER v.6. Hierarchical cluster analysis was performed on log-transformed density or presence/absence data. ANOSIM was used to detect differences between groups in each analysis, while the SIMPER routine was used to determine the relative contribution of key species to the similarity or difference between estuary, mangrove presence/absence or seasonal groups.

A significance level of $P < 0.05$ was used in all analyses. All analyses were performed using R-studio with packages mgcv, MASS, VEGAN, and qqplot 2 (Team 2013; Studio 2012; Wickham and Chang 2009; Oksanen et al. 2007; Wood and Wood 2007) unless otherwise specified.

3.4. RESULTS

3.4.1. Environmental and biological variability

Salinity and dissolved oxygen varied significantly ($P < 0.001$) among estuaries. Greater freshwater inputs at Qora and Xhora resulted in lower mean summer values of 28.6 and 30.0, respectively, while values at Nahoon (32.6) and Gonubie (32.7) remained high. Low rainfall during winter resulted in little variance amongst winter salinity profiles. Dissolved oxygen levels were typically lower at Gonubie and Xhora with mean values of 7.6 and 7.8 mg/l, respectively, than the comparably high values recorded at Nahoon (8.0 mg/l) and Qora (8.9 mg/l). No significant difference in temperature was found among estuaries however average values at Qora and Xhora were higher in summer at 23.9 and 23.6°C, respectively, than the southern estuaries at 20.4 and 20.5°C for Nahoon and Gonubie, respectively. Temperature, salinity and turbidity ($P < 0.001$) varied significantly by season (Table 3.2). No recorded environmental variables showed significant variation between estuaries with and without mangroves.

Species richness and Shannon’s diversity index varied significantly among estuaries where average values of both indices were higher in the two northern estuaries than those of the southern estuaries. Density (total number of individuals m^{-3}) was not significantly different amongst estuaries although average values were highest at Qora (6.19 m^{-3}) and lowest at Gonubie (3.3 m^{-3}). There was no significant relationship between any of the selected univariate indices and the presence or absence of mangroves within estuaries.

The relationship between total density of early stage fishes per site and environmental variables was significant within estuaries where higher temperatures ($P < 0.001$) and lower salinities ($P < 0.05$) played a positive role in early stage fish density. Temperature was found to significantly ($P < 0.05$) influence species richness while Shannon’s index was significantly ($P < 0.01$) influenced by salinity.

Table 3.2: Average (range) temperature (°C), salinity, turbidity (NTU) and dissolved oxygen (mg/l) measurements recorded during summer and winter of 2015 and 2016.

Estuary	Environmental variable	Season	
		Summer (range)	Winter (range)
Nahoon	Temperature	20.4 (16.5 – 25.1)	18.1 (16.0 – 19.6)
	Salinity	32.6 (29.8 – 35.3)	34.4 (32.4 – 35.7)
	Turbidity	3.6 (0 – 9.4)	2.8 (0 – 4.3)
	Dissolved oxygen	7.9 (3.4 – 9.3)	8.0 (6.4 – 11.1)
Gonubie	Temperature	20.5 (14.3 – 25.8)	17.6 (14.2 – 19.1)
	Salinity	32.7 (30.4 – 35.0)	34.4 (30.3 – 35.7)
	Turbidity	5.7 (0 – 13.2)	1.4 (0 – 5.0)
	Dissolved oxygen	7.4 (5.8 – 8.6)	7.9 (7.2 – 8.6)
Qora	Temperature	23.9 (17.3 – 28.0)	16.7 (14.5 – 18.2)
	Salinity	28.6 (19.4 – 34.7)	34.0 (31.5 – 35.7)
	Turbidity	4.7 (1.2 – 9.2)	1.2 (0 – 6.2)
	Dissolved oxygen	9.4 (5.8 – 12.5)	8.3 (7.5 – 9.1)
Xhora	Temperature	23.6 (21.6 – 25.0)	17.6 (15.9 – 19.6)
	Salinity	30.0 (25.8 – 33.9)	33.9 (32.4 – 35.7)
	Turbidity	4.6 (2.1 – 8.6)	1.3 (0 – 4.2)
	Dissolved oxygen	7.7 (5.4 – 9.8)	8.0 (6.9 – 9.4)

3.4.2. Species composition and association

A total of 12 597 early stage fishes from 23 families and representing 47 species were caught in this study spanning two summer and winter seasons over two consecutive years. Estuaries with mangroves yielded 5 815 fishes with the remaining 6 782 fishes coming from the Gonubie and Qora. Clupeidae were the dominant fish family, represented predominantly by a single estuarine species, *Gilchristella aestuaria*, which comprised 51% of the total catch. The family Gobiidae with eight species was the second most abundant contributing 40% of total catch and with only two species, *Caffrogobius gilchristi* and *Glossogobius callidus*, contributing more than 70% and making up nearly 30% of total catch. The third most abundant family was the Blennidae contributing nearly 6% toward total catch, predominantly represented by *Omobranchus woodi*. All other fish families contributed less than 1% (Table 3.3).

Of the 47 species of early stage fishes captured, 28 species were positively identified to species level. This assemblage represented the majority at 88% of the total catch. The estuarine-spawned category was represented by 10 species and dominated the catch of identified species comprising 97% of the total, owing to the particularly high abundances of *Gilchristella aestuaria, Caffrogobius gilchristi* and *Glossogobius callidus*. The marine-spawned category was represented by 18 species but two of these species alone, *Rhabdosargus holubi* and *Monodactylus falciformis,* comprised nearly 80% of their total numbers. Unidentified species occurred in low numbers and typically near to the estuary mouth, it is assumed therefore that most of these species were marine stragglers entering estuaries incidentally via tidal exchange.

3.4.3. Temporal and spatial trends in fish density

Densities of early stage fishes showed a clear difference between summer and winter and were significantly ($P < 0.001$) higher in summer than winter which was consistent between estuaries with ($P < 0.01$) and without ($P < 0.001$) mangroves. Individual analysis of each estuary indicated that there was no difference in densities between summer and winter for the Nahoon Estuary and while significant, seasonal densities varied only marginally in the Gonubie Estuary. This low variance between seasons is explained by the high winter densities of *Caffrogobius gilchristi* replacing the high summer densities of *Gilchristella aestuaria*. The two northern estuaries, Qora and Xhora, however displayed large variance between summer and winter densities of early stage fishes (Figure 3.2) with particularly high summer densities of *Gilchristella aestuaria* and *Glossogobius callidus* at Qora.

Spatial variation amongst sampling sites was evident with highest densities typically found at three to four kilometres from the estuary mouth, where the lowest densities were typically recorded. Variation in early stage fish densities among sampling sites was only significant ($P < 0.01$) for estuaries without mangroves. Estuaries with mangroves showed no significant variation in early stage fish densities per site. Estuarine use categories showed dissimilar spatial density distributions within the study estuaries. Marine-spawned species showed no significant differences in density per site. Estuarine-spawned species however differed significantly ($P < 0.01$) in density per site in all estuaries except for Xhora where densities were distributed evenly amongst sampling sites (Figure 3.3).

Table 3.3: Species composition, mean density, percentage of total catch, estuarine usage functional group (Potter et al. 2015) and presence/absence among estuaries sampled for early stage fishes caught during winter and summer of 2015 and 2016.

Family	Species	Mean Density (100 m^{-3})	% total catch	Body length (mm)		Functional group	Presence/Absence			
				Mean	Range		Gon	Nah	Qor	Xho
Ambassidae	Ambassid sp. 1	0.81	0.18	8.8	6.9-10.8	EM	■	■	■	■
Atherinidae	*Atherina breviceps*	0.27	0.06	6.4	4.3-13.6	EM	■	■	■	■
	Atherina sp. 1	0.04	0.01	5.6		-				■
Blenniidae	Bleniid sp. 1	0.03	0.01	6.9		-			■	
	Bleniid sp. 2	7.34	1.63	2.3	1.5-12.1	-	■	■	■	■
	Omobranchus woodi	16.98	3.77	3.1	1.7-13.6	EM	■	■	■	■
Bregmacerotidae	*Bregmaceros* sp. 1	0.02	0.00	5.5		MS				■
Cheilodactylidae	*Cheilodactylus* sp. 1	0.07	0.02	9.1		EM			■	
Clupidae	*Etrumeus whiteheadi*	0.4	0.09	8.5	4.2-16.5	MS		■	■	■
	Gilchristella aestuaria	229.68	51.01	17.5	2.6-24.0	SE	■	■	■	■
Eleotridae	Eleotrid sp. 1	0.03	0.01	12.9		-			■	
Elopidae	Elopiform sp. 1	0.17	0.04	32.2	31.0-35.1	-	■			
Engraulidae	*Engraulus japonicus*	0.05	0.01	15.5	12.0-19.0	MS	■		■	
	Stolephorus holodon	0.07	0.02	25.5	24.0-27.0	MEO			■	■
Gobiesocidae	*Chorisochismus dentex*	0.03	0.01	4.8		MS			■	
Gobiidae	*Caffrogobius gilchristi*	65.53	14.55	2.7	1.8-5.9	EM	■	■	■	■
	Caffrogobius nudiceps	11.09	2.46	2.8	2.0-4.5	EM	■	■	■	■
	Caffrogobius sp. 1	0.03	0.01	1.7	1.7-1.8	-			■	
	Glossogobius callidus	59.64	13.25	11.2	2.1-18.0	EM	■	■	■	■
	Gobiidae sp. 1	11.17	2.48	3.3	2.1-4.6	-	■	■	■	■
	Gobiidae sp. 2	33.82	7.51	3.6	2.4-4.6	-	■	■		■
	Psammogobius knysnaensis	1.64	0.36	2.3	1.8-5.6	EM	■	■	■	■
	Redigobius dewaali	0.67	0.15	2.1	1.8-2.3	EM			■	■

Family	Species	Mean Density (100 m^{-3})	% total catch	Body length (mm)		Functional group	Presence/Absence			
				Mean	Range		Gon	Nah	Qor	Xho
Haemulidae	*Pomadasys commersonnii*	0.03	0.01	14.4		MED			■	
	Pomadasys olivaceum	0.02	0.00	12.0		MS				■
Kyphosidae	*Neoscorpis lithophilus*	0.08	0.02	13.6	13.2-14.0	MS		■		
Leiognathidae	*Leiognathus equulus*	0.04	0.01	9.6		EM			■	
Monodactylidae	*Monodactylus argenteus*	0.03	0.01	16		MEO			■	
	Monodactylus falciformis	2.14	0.48	5.7	2.1-18.0	MED	■	■	■	■
Mugilidae	*Liza* sp. 1	0.82	0.18	10.3	7.2-17	-	■	■	■	■
	Mugil cephalus	0.09	0.02	20.6	16.3-24.0	MED	■			■
	Myxus capensis	0.1	0.02	12.2		CA		■		
Sciaenidae	*Umbrina* sp. 1	0.03	0.01	3.6		MS		■		
Serranidae	*Epinephelus* sp. 1	0.02	0.00	5.8		MS				■
Sillaginidae	Sillaginid sp. 1	0.08	0.02	9.5	5.0-11.4	-				■
	Sillaginid sp. 2	0.24	0.05	2.3	1.7-3.6	-				■
Soleidae	*Heteromycteris capensis*	0.47	0.10	2.2	2.0-3.2	MEO		■	■	
	Solea turbynei	0.35	0.08	3.3	1.1-4.2	MEO		■	■	■
Sparidae	*Acanthopagrus vagus*	1.26	0.28	6.2	4.9-8.5	MED			■	■
	Rhabdosargus globiceps	0.03	0.01	9.8	6.8-12.0	MEO				■
	Rhabdosargus holubi	4.42	0.98	10.2	7.8-12.5	MED	■	■	■	■
	Sarpa salpa	0.2	0.04	12.8	11.1-14.3	MEO	■	■	■	
Syngnathidae	*Syngnathoides biaculeatus*	0.1	0.02	11.0		EM		■		
	Syngnathid 1	0.04	0.01	9.0		-				■
	Syngnathid 2	0.02	0.00	4.5		-			■	
Tripterygiidae	*Helcogramma* sp. 1	0.04	0.01	9.1		MS	■		■	■
Unidentified	Unidentified sp. 1	0.04	0.01	9.8		-		■		

Functional group: MS marine stragglers, MEO marine-estuarine opportunist, MED marine-estuarine dependent, EM estuarine migrant, SE solely estuarine, CA catadromous species

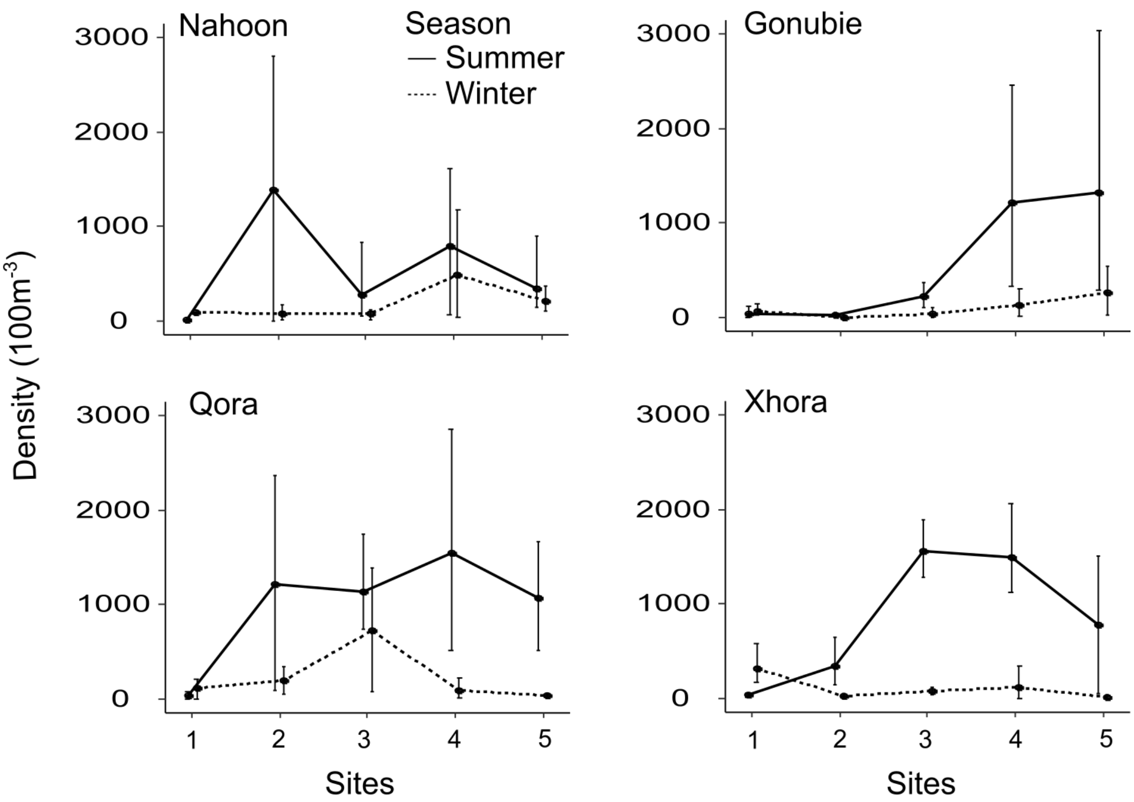

Figure 3.2: Total density for all species of early stage fishes captured per site from study estuaries during summer and winter in 2015 and 2016, bars indicate range (min – max).

3.4.4. Temporal and spatial trends in species richness and diversity

No seasonal differences in species richness or diversity were observed for either estuaries with or without mangroves. Analysis of individual estuaries by season similarly showed no significant variation in species richness with similar numbers of species encountered throughout the year in the two southern estuaries and a higher but similar number of species occurring in the two northern estuaries. Owing to the particularly high summer densities of *Gilchristella aestuaria* and *Glossogobius callidus* in the Qora Estuary, diversity among seasons varied significantly for this estuary alone. Species richness and diversity were not significantly different among sampling sites for either estuaries with or without mangroves which was consistent per estuary despite higher species numbers typically being encountered near the estuary mouths.

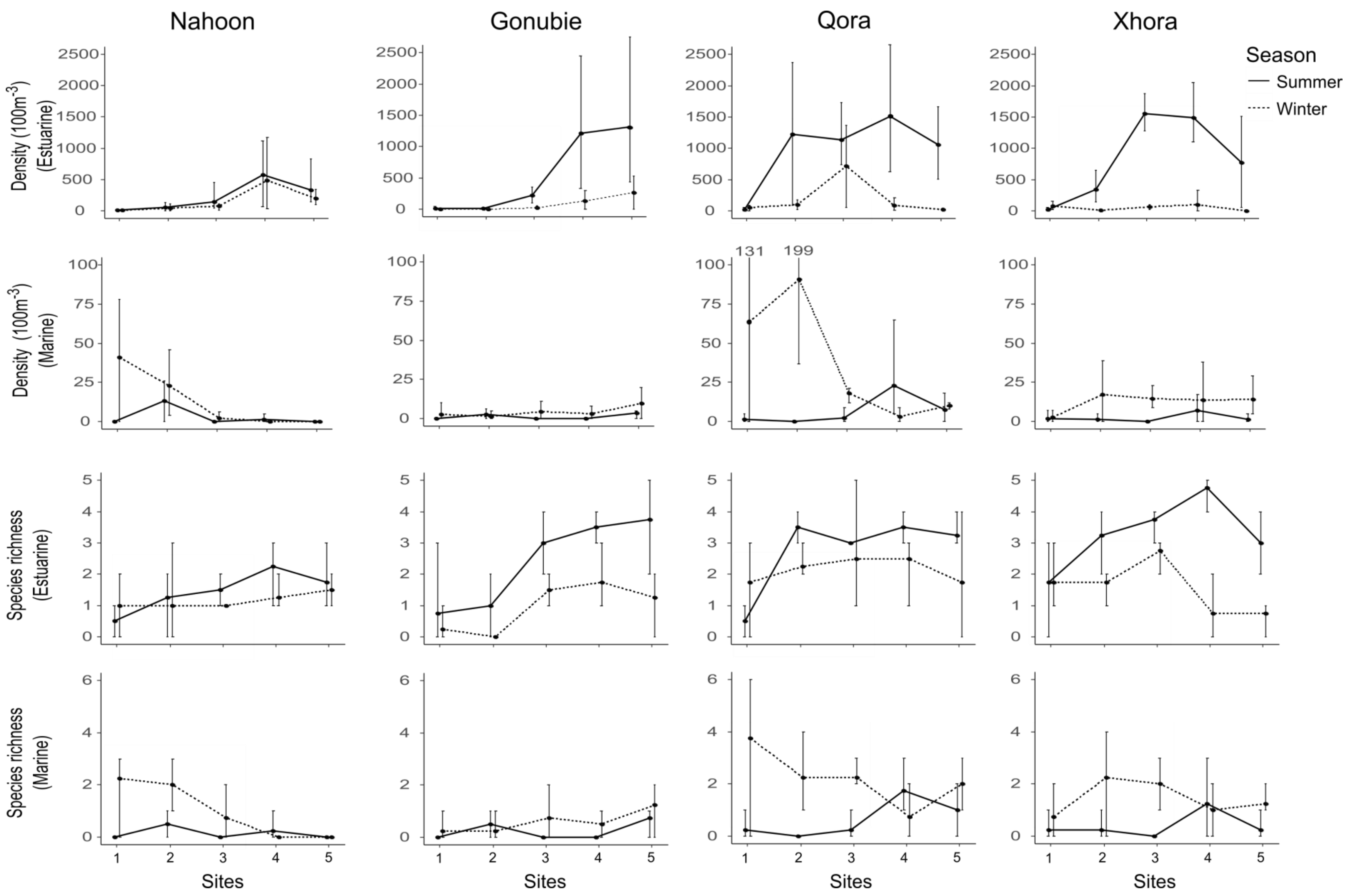

Figure 3.3: Horizontal trends in early stage fish density and species richness of estuarine- and marine-spawned categories per sampling location from study estuaries during summer and winter of 2015 and 2016, bars indicate range (min – max).

3.4.5. Spatial distribution and environmental factors

Using Generalised Additive Models an optimal model describing total density was produced with salinity, temperature and turbidity as independent variables (Table 3.4). Densities were predicted to increase along with the range of observed declining salinities while increasing but peaking at intermediate summer temperatures. Highest densities were also predicted to occur at low turbidity values but not in clear water (Figure 3.4). Estuarine- and marine-spawned species analysed separately produced better model fits with observed variables while still typically responding similarly. Densities of estuarine-spawned species were significantly driven by temperature, turbidity and salinity where densities were predicted to be highest at higher temperatures, low turbidity's and low observed salinities. Densities of marine-spawned species were significantly influenced by salinity, temperature and season, where density increased with declining salinity. Temperature had a bimodal effect which reflected upper and lower temperatures of winter and summer, respectively, as seasonality was additionally a significant variable in explaining density of marine-spawned fishes.

Species richness for all combined taxa is predicted to increase linearly with temperature and water clarity, although the model poorly fitted the data explaining little of the observed variance. Individual analyses of estuarine-usage categories improved prediction of species richness with observed variables indicating independent group responses (Figure 3.5). Estuarine-spawned species richness is predicted to respond positively to increased temperatures and lower salinities. Marine-spawned richness is predicted to increase similarly with lower salinities but additionally with water clarity while also being significantly affected by season with higher species numbers expected in winter.

Table 3.4: Generalised Additive Model results for early stage fish density versus environmental variables of all taxa, estuarine usage categories and dominant species recorded during summer and winter sampling of study estuaries (2015 – 2016).

Grouping (no. species)	Deviance explained (%)	Significant variable
Density		
All taxa (47)	37	temp*** NTU***sal*
Estuarine (10)	39	temp***NTU***sal***
Marine (18)	46	se***temp***sal***
Species richness		
All taxa	6	sal**NTU*
Estuarine	15	temp*sal***
Marine	32	se***sal***NTU***
Dominant species		
Gilchristella aestuaria	70	temp***pH***do***
Caffrogobius gilchristi	60	temp***sal***ntu***
Glossogobius callidus	89	NTU***ph***do***TDS***
Rhabdosargus holubi	34	se***NTU*
Monodactylus falciformis	13	sal**temp*

temp temperature; *NTU* turbidity; *se* Season; *sal* salinity; *do* dissolved oxygen; *TDS* total dissolved solids

*(significance codes ***P <0.001; ** <0.01; * <0.05)*

3.4.6. Community analysis

A cluster analysis of estuaries based on early stage fish presence/absence and density showed no separation between estuaries with and without mangroves. Estuaries were however rather separated into northern (Qora and Xhora) and southern (Nahoon and Gonubie) groups based on both variables (Figure 3.6), although this relationship was not significant. High densities of *Glossogobius callidus, Redigobius dewaali, Atherina breviceps* and *Psammogobius knysnaensis* in the northern estuaries contributed to this dissimilarity between groups for estuarine-spawned species. Higher densities of the marine-spawned *Acanthopagrus vagus, Heteromycteris capensis* and *Stolephorus holodon* contributed to dissimilarity between north and south groups. All estuaries were significantly (*P* < 0.05) separated by season based on densities of early stage fishes, this was primarily attributed to the high summer densities of *Gilchristella aestuaria, Caffrogobius nudiceps, Glossogobius callidus* and *Omobranchus woodi* (SIMPER).

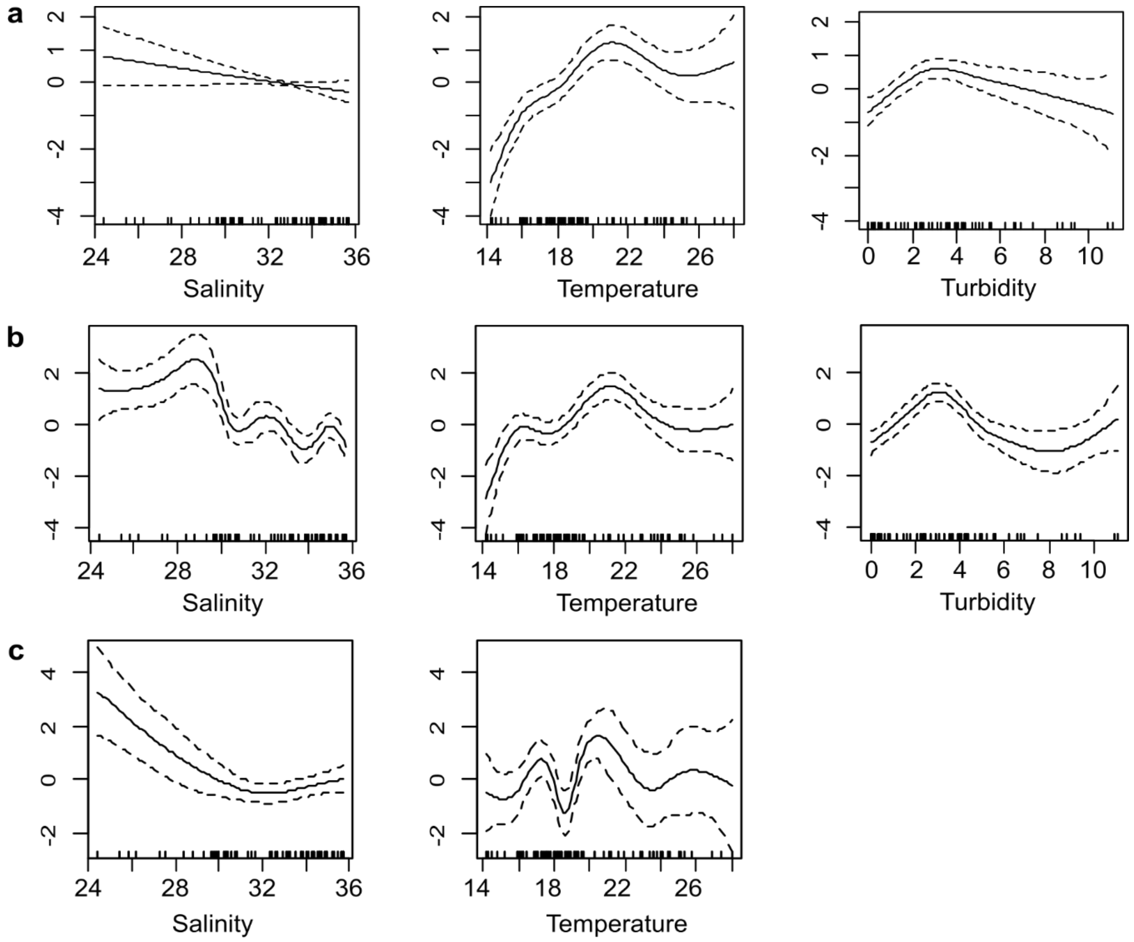

Figure 3.4: Results of GAM's showing effect of significant environmental variables on density of (a) all taxa, (b) estuarine- and (c) marine-spawned species for fishes captured during summer and winter of 2015 and 2016 (dotted lines indicate 95% confidence intervals).

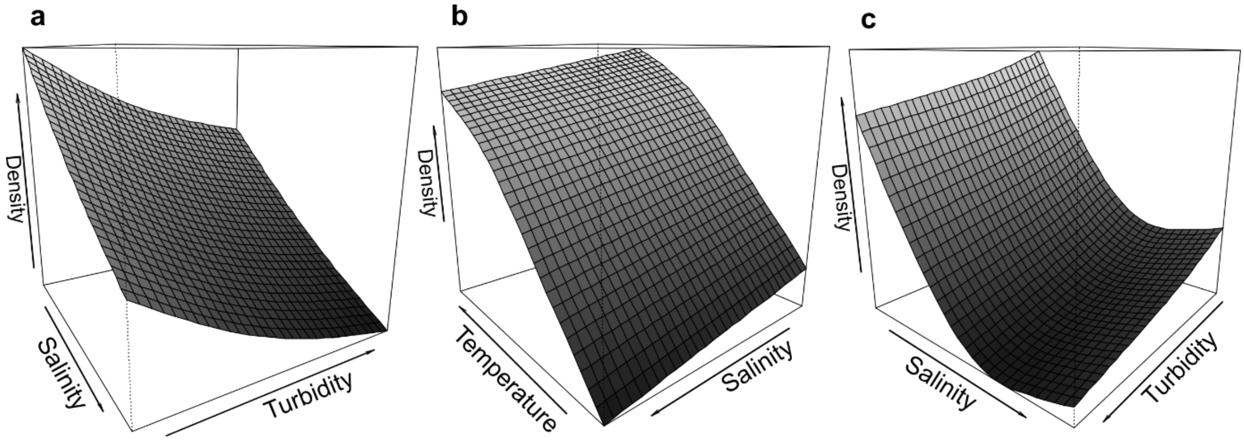

Figure 3.5: GAM plots of significant environmental variables on species richness for (a) all taxa, (b) estuarine- and (c) marine-spawned species captured during summer and winter of 2015 and 2016.

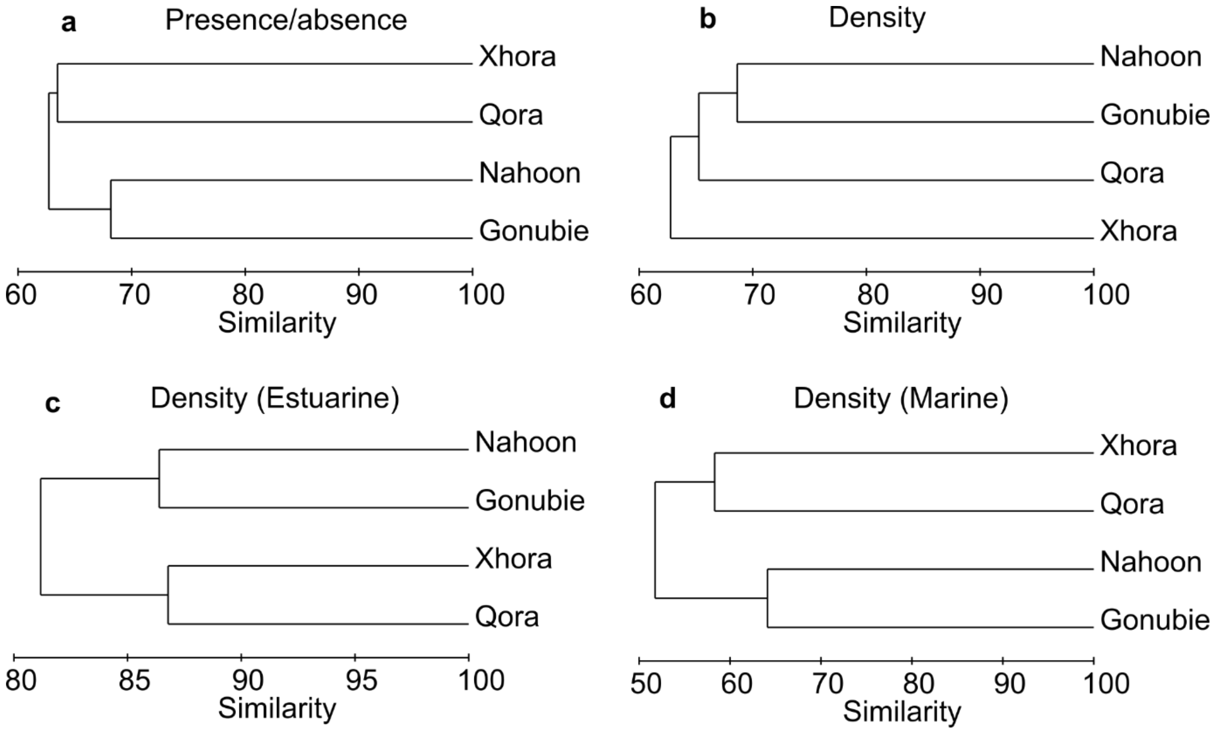

Figure 3.6: Bray-Curtis similarity dendrogram showing classification of sampled estuaries based on (a) presence/absence and density for (b) all, (c) estuarine- and (d) marine-spawned early stage fishes captured during summer and winter of 2015 and 2016.

3.5. DISCUSSION

This study found that the presence of mangroves within warm temperate South African estuaries had no significant effect on the density or richness of early stage fish communities inhabiting these systems. This observation was consistent when comparing estuarine and marine usage categories separately. Additionally, no species were found to be unique to mangrove estuaries and there was no dissimilarity of early stage fish communities from estuary groups with or without mangroves. It is frequently observed that environments with increased habitat diversity have a higher diversity of animal communities than more uniform systems (Messmer et al. 2011, Whitfield 1999). This is attributed to the fact that a greater number of habitats increases the opportunities for species to specialise on different resources and coexist (Tews et al. 2004), with some habitats often having a disproportionate impact on communities where greater density and richness may be credited to greater survival, growth or larval supply (Messmer et al. 2011, Beck et al. 2001). Tropical mangrove forests provide such a habitat where their presence is linked to a greater abundance and richness of species (Nagelkerken et al. 2001, Robertson and Duke 1987) while forest area has been shown to positively relate with fisheries production (Aburto-Oropeza et al. 2008; Manson et al. 2005). Submerged mangrove roots provide a sheltered, structurally heterogenous area where high densities of zooplankton, infauna and meiofauna are often recorded (Kathiresan 2001), with particularly high densities of brachyuran zoea (Kathiresan 2001, Robertson et al. 1988).

Climatic, oceanic and hydrodynamic conditions vary greatly along the South African coastline potentially making cross estuary comparisons difficult as each system presents a unique set of conditions, with these variable conditions strongly influencing fish communities (Strydom 2015). However, general trends and similarities among systems are typically found in estuaries of the same climatic zone, rainfall pattern, river inflow and temperature (Strydom 2015, Strydom 2002, Whitifield 1999). While significant variation was observed in salinities of the study estuaries, this was confined to dissimilarity between the grouped northern (Qora and Xhora) and southern (Nahoon and Gonubie) systems with no difference within the groups. Multivariate analysis of the fish communities reflected this observation where both presence/absence and density were separated into northern and southern groups with no dissimilarity between estuary groups with and without mangroves being evident. Dissimilarity of fish communities between northern and southern groups is attributed primarily to higher freshwater inputs of the northern estuaries and secondarily to latitudinal affects with more species being represented in northern estuaries.

Temperature, turbidity and axial salinity gradient appeared to be far greater determinants of inhabiting fish communities than did increased habitat heterogeneity offered by the presence of mangroves. Several studies have shown that these same factors influence the monthly variation in abundance of early stage fishes recruiting into estuaries (Pattrick and Strydom 2014; Whitfield 2005; Neira et al. 1992). Temporal communities further being strongly driven by seasonal rhythmicity in reproduction and recruitment which, in temperate systems, is particularly apparent (Strydom 2015; Maes et al. 1998). While a salinity gradient was typically not well established within the sampled locations of the estuaries under study, with minimum values not reaching below 20, decreases in salinity associated with freshwater inputs or runoff were consistently and significantly related to increases in species richness and density. Generalised Additive Models typically revealed a linear response of increasing total density to declining salinity, within the limited range of observed variation of salinity, while responses to temperature or turbidity tended to be modal. As is commonly found among estuarine studies, marine estuarine-dependent species showed a particularly strong response to freshwater inputs with sharp increases in densities (Whitfield 1994). This is attributed to increased recruitment as a direct result of cueing responses and/or food availability via increased productivity (Whitfield 2005; Strydom et al. 2003).

Community responses of early stage fishes to turbidity are likewise similarly reported with increases in fish density attributed to the refuge provision afforded by more turbid waters (Whitfield 1999; Blaber and Blaber 1980). The current results showed that total density as well as species richness increased with NTU although densities peaked rapidly at low turbidity's. Increased feeding success in suspensoid-rich waters is another factor attributed to increased densities within turbid waters but this may be inhibited at high levels (Whitfield 1999). Sampling for early stage fishes in the current study was performed at night and in the upper water column to account for diel vertical migrations, which have been shown to correspond closely with the vertical distribution of prey (Neilson and Perry 1990; Fortier and Leggett 1983). Distributions may therefore reflect areas of high bottom and low surface turbidity where refuge and feeding may be maximised along with tidal and diel vertical migration. This may be evidenced by the highest densities of estuarine-spawned species being found approximately 3–4 kilometres from the mouth and not at regions where the lowest salinities were found and nearer to mesohaline zones where the highest productivity is typically recorded (Strydom et al. 2003), although this zone lay outside of the sampling region in the current study due to generally low freshwater inflow.

Densities of early stage fishes showed a bimodal response to temperature with seasonal peaks corresponding to winter and summer temperatures of approximately 16 and 22°C, respectively. Much of the winter density peak is attributed to *Caffrogobius gilchristi* which dominated catches with 65% of the total catch alone, adult spawning appearing to favour cooler waters with winter catches as much as eight times higher than those of summer. Pattrick and Strydom (2014) recorded highest densities of *C. gilchristi* in summer and autumn of Eastern Cape estuaries, as did Pattrick et al. (2007) in a subtropical system, concluding that recruitment was likely protracted into autumn due to prevailing high temperatures. Conversely, Wasserman et al. (2010) recorded highest densities in winter with temperatures similar to those of the current study. It cannot be determined why such disparity in catches would occur between studies within the same climatic area although winter production would reduce larval competition with the other highly abundant estuarine species such as *Gilchristella aestuaria* and *Glossogobius callidus*.

In conclusion, it was anticipated that among otherwise similar temperate estuaries the addition of a structurally heterogenous habitat of mangroves would have some impact on the inhabiting fish communities by providing refuge which is rich in food resources. Tropical mangroves support a range of species which inhabit these regions either temporally during ontogenetic phases, often as juveniles, or permanently throughout their life-cycle. The presence and size of these habitats are often related to the success and eventual biomass of adult stocks either through their nursery value as proposed by Beck et al. (2001) or by the seascape nursery and its interconnectivity as per Nagelkerken et al. (2015) and ultimately relating to greater larval supply. Instead it appears that for warm temperate estuarine systems the effects of seasonality, freshwater inputs and its combined impact on phytoplankton and zooplankton production result in an overarching effect on densities of inhabiting fish communities whose success is limited by these factors rather than additional habitat provision. Whitfield (1990) put forward that South African estuaries have all the features of an unpredictable environment, being regions with short lived periods of stability and high environmental gradients. As such, inhabiting species need to be robust and adaptable, which is exemplified by species which use estuaries as juveniles and migrate to marine environments when adults, which would explain the absence of any warm temperate mangrove-dependent species. Variation in abundance, as shown above, relates strongly to effects of freshwater inputs and it is likely this feature which provides the limiting factor on early stage fish densities.

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CHAPTER 4

EVIDENCE FOR HABITAT RESIDENCY AND ISOTOPIC NICHE PARTITIONING IN A MARINE ESTUARINE-DEPENDENT SPECIES ASSOCIATED WITH MANGROVE HABITATS ON THE EAST COAST OF SOUTH AFRICA

(This manuscript has been submitted to the journal *Estuaries and Coasts* and has been accepted with minor changes)

4.1. SUMMARY

Estuaries are valuable fish nurseries due to an abundance of food and the provision of shelter in a spatially heterogeneous area. Habitats within estuaries vary in their specific supply of these two factors with more structurally complex habitats tending to offer more of both food and refuge. In this study, we investigated the site residency of an abundant estuarine-dependent sparid, *Rhabdosargus holubi*, from two nearby but dissimilar mangrove habitats using two approaches, mark-recapture and stable isotope analysis. Results showed that rates of residency for both short- (in the order of days) and long-term (weeks) were high. Stable isotope ratios (carbon and nitrogen) were used to compare the isotopic niche of two size groups between the two mangrove habitats. Both small and larger juveniles from a more complex creek associated with red mangroves and seagrass had a significantly broader isotopic niche width when compared with the groups from a more homogenous white mangrove habitat. *Rhabdosargus holubi* appear to establish strong residency at sites soon after recruitment which may persist until their departure from the estuary despite apparent differences in habitat quality.

4.2. INTRODUCTION

Estuaries are valuable nursery areas for the juveniles of many marine spawned species which, together with high levels of primary and secondary productivity, translates into ecosystems which are both ecologically and economically important. Migrations of nekton between estuaries and the marine environment contributes to a broad energy transfer across these systems (Carassou et al. 2016; Gillanders et al. 2003). This is particularly true for marine spawned species which enter estuaries as late-stage larvae or juveniles, gain biomass in these habitats, and later move to adult habitats where they contribute directly or indirectly to fisheries production (Manson et al. 2005; Barbier and Strand 1998; Boehlert and Mundy 1988).

Movements of young fishes between nursery and adult habitats is often inferred from size frequency distributions associated with specific habitats (Cocheret De La Morinière et al. 2003), however little can be inferred about movement of juvenile fishes within specific habitats using this method. Knowledge of the movements of fishes within estuaries, causes and mechanisms, habitat preferences and residency is essential to understanding how fishes use these areas as nurseries (Beck et al. 2001). The relative nursery value of estuarine habitats clearly differs where structurally more complex habitats are often found to provide better feeding opportunities, reduced predation or both (Laegdsgaard and Johnson 2001). Habitat heterogeneity within nursery areas also provides opportunities for a broader feeding niche (Quevedo et al. 2009; Layman et al. 2007a), which may be particularly valuable for juveniles needing to sustain high rates of growth. For example, Irlandi and Crawford (1997) found that intertidal saltmarsh creeks adjacent to seagrass beds were found to have higher densities and growth rates for the Western Atlantic pinfish *Lagodon rhomboides* (Family Sparidae)*,* than marsh creeks adjacent to unvegetated areas*.*

Movement of fishes within estuaries has typically been assessed by repeated sampling within various habitats where size frequencies and densities are used as proxies for movement and use (Beck et al. 2001; Irlandi and Crawford 1997; Claridge et al. 1986). More recently, technological advancements have allowed greater accuracy in movement studies. Acoustic telemetry has been successfully used in estuaries to provide information on area use, residency and movement of older juveniles and adults of species in Paralichthydae, *Paralicthys dentatus* (Szedlmayer and Able 1993), Sciaenidae, *Argyrosomus japonicas*

(Cowley et al. 2008), and Sparidae, *Lithognathus lithognathus* (Bennett et al. 2012). Acoustic telemetry studies are however restricted to larger sized individuals neglecting smaller juveniles and smaller species, many of which may contribute significantly to biomass and trophic connectivity among habitats. Mark-recapture studies using relatively benign techniques, such as Visible Implant Elastomer (VIE), provide an opportunity for the assessment of habitat use and residency for smaller sized fishes. VIE tags have been successfully used in a variety of field and laboratory studies on fishes from as small as 8 mm in estuaries (Bushon et al. 2007; Skinner et al. 2005) and marine environments (Brennan et al. 2005; Frederick 1997).

Stable isotopes have also been widely used to determine movements or residency in fishes (Gray et al. 2004; Cocheret De La Morinière et al. 2003) as stable isotopes of carbon enable determination of food sources consumed by an organism as well as the habitat where the consumer found its food (Layman et al. 2007a; Post 2002). Mobile species will therefore tend to assimilate resources from various areas and thus become more isotopically homogenous (Hansson et al. 1997) while species exhibiting high site residency will tend to show site specific signatures (Harrod et al. 2005; Gray et al. 2004). By combining stable isotope analysis with mark-recapture data, valuable insight can be gained on temporal movement (Cunjak et al. 2005), dietary trophic coupling and isotopic niche partitioning among subpopulations (Pimiento et al. 2015; Layman et al. 2007b; Newsome et al. 2007).

Rhabdosargus holubi (Steindachner, 1881) is one of the most common and widely distributed fishes in South African estuaries which, owing to its high abundance, is responsible for huge transfers of biomass among aquatic habitats during ontogenetic migrations (Carassou et al. 2016; Cowley and Whitfield 2001). Juveniles are dependent on estuaries while adults are found in nearshore marine environments where spawning takes place (Heemstra and Heemstra 2004; Blaber 1973). Postflexion larvae migrate into estuaries, presumably guided by olfactory cues (James et al. 2008; Strydom et al. 2003; Boehlert and Mundy 1988), reside in the estuary as juveniles and then return to the ocean as sub-adults. Juveniles are widely tolerant to changes in both salinity and temperature (Blaber 1973).

Postflexion *R. holubi* feed almost exclusively on copepods (Carassou et al. 2016), whereas juveniles from 30 mm upwards feed mainly on aquatic macrophytes, filamentous algae and epibenthic invertebrates (De Wet and Marais 1990). While aquatic vegetation often forms the bulk of their gut contents, *R. holubi* assimilate little plant material due to a lack of cellulase and indirectly target the epiphytic diatoms and bryozoa (Carassou et al. 2016; Blaber 1974). An ontogenetic shift in diet occurs with the onset of maturity (ca. 17 cm TL at two years of age) accompanied by a change in dentition from tricuspid to molariform facilitating the adult diet of molluscs, crustaceans and polychaetes (Blaber 1974).

Movement studies on this important species in estuaries have been precluded by its small size during the nursery phase and use patterns were inferred using traditional methods. The aim of the present study was to use VIE marking coupled with stable isotope analysis to further understand the habitat use of this species during the nursery phase. A mangrove estuary on the east coast of South Africa was selected as a study area for testing small-scale movement patterns where different species of mangrove provide varying levels of shelter and food availability in close proximity to each other. The objectives were to determine: (1) short term rates of residency for *Rhabdosargus holubi* associated with different mangrove root types, and (2) the degree to which these fish share habitat space using size specific isotopic signatures. It was anticipated that residency would be lower and isotopic niche narrower at the more homogenous white mangrove site compared to the eelgrass fringing red mangrove habitat, with the latter providing more structural complexity and wider feeding opportunities.

4.3. MATERIALS AND METHODS

4.3.1. Study site

The permanently open Mngazana Estuary enters the Indian Ocean just south of Port St. Johns (31°42’ S 29°25’ E) on the subtropical east coast of South Africa and is approximately 5.3 km long (Figure 4.1). The catchment of 275 km^2 lies mostly in the coastal hills where it feeds the 150 km long river (Branch and Grindley 1979). The Mngazana Estuary has the third largest mangrove forest in South Africa, covering an area of 118ha (Rajkaran and Adams 2012) where three species of mangrove co-occur, namely, white *Avicennia marina,* red *Rhizophora mucronata*, and black *Bruguiera gymnorrhiza*.

White mangroves play a pioneering role where saplings occur in areas of active accretion and they are well established as groves along the Mngazana main channel (Branch and Grindley 1979). Conversely, red mangroves tend to dominate the fringes of the shallow mangrove creeks where this species excludes other mangroves. The eelgrass *Zostera capensis* commonly also occurs in these sheltered shallow channels (Branch and Grindley 1979).

Sampling was performed at two sites in the lower Mngazana Estuary. Site WM (white mangrove) was a 60 m region of shoreline along the main channel with a monospecific stand of white mangroves. Just beyond the intertidal reach of the mangrove pneumatophores, the gradient increased rapidly dropping to water depths > 2 m. Site RM (red mangrove) was located at a small embayment with a near-monospecific red mangrove stand spanning a shoreline of approximately 60 m. The embayment was shallow with depths < 1.5 m and dense fringing eelgrass beds. The two sampling sites were both situated in the lower reaches of the estuary approximately 500 m apart. Physico-chemical measurements of salinity (psu), temperature (ºC), turbidity (NTU) and dissolved oxygen (ms.cm^{-1}) were taken at the time of each sampling event with a YSI-6600 multiparameter probe.

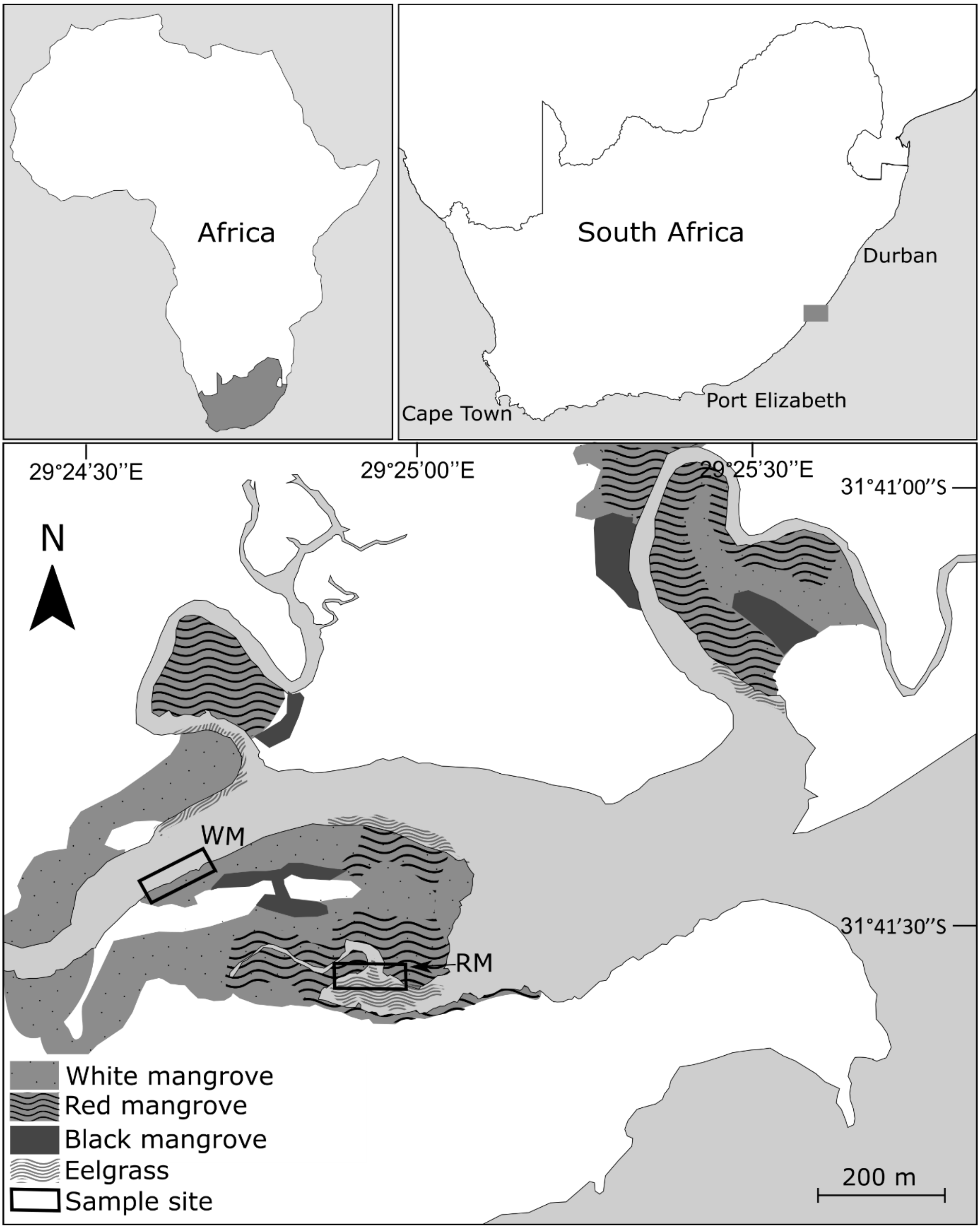

Figure 4.1: Location of Mngazana Estuary along the east coast of South Africa. Dominant mangrove type and sampling sites indicated.

4.3.2. Mark-recapture

Silicon-based Visible Implant Elastomer (VIE) tags (Northwest Marine Technology, Inc., Shaw Island, Washington, USA) were subcutaneously injected with an insulin syringe (0.33 mm/29-gauge hypodermic needle) to mark all fishes. Two colours (red and green) and four body locations (right and left dorsal margin and caudal peduncle) allowed for up to eight individual group markings. Colours and body regions were alternated each day to determine the date of marking of individuals upon recapture. VIE marking has been proven to be a reliable, long-lasting and low impact method for a variety of fish species (Bangs et al. 2013; Reeves and Buckmeier 2009; Brennan et al. 2007; Brennan et al. 2005).

Marking took place in summer of 2015 and 2016 over five consecutive days. Fish were captured daily using a 50 m seine net (2 m depth, 12 mm stretched mesh) which was deployed from a boat to sweep an area of approximately 400 m^2. Only *R. holubi* were retained from catches and kept in submerged pop-up mesh nets (350 x 350 x 400 mm with 5 mm mesh). Seine hauls were repeated until approximately 50 individuals were obtained at each site per day which ranged from one to six seine hauls. All marked and recaptured fish were measured in total length (TL) and released into the submerged mesh nets in the water to monitor survival prior to release. Fish in ill health or those that did not survive the procedure were removed from the experiment, however this was minimal. All marked fish were released at the site of capture once marking was completed. The following day the same locations were resampled until the target of 50 *R. holubi* at each site was attained. Marked fish were counted and measured and unmarked fish were kept separate in the keep nets, after which they were also measured and marked. All marks were recognised by visual observation.

4.3.3. Stable Isotope Analysis

On the final day of the 2015 mark-recapture study, unmarked individuals from each site were retained for stable isotope analysis. All individuals were euthanized immediately by immersion in a container filled with ice and frozen for later processing. In the laboratory, samples were thawed, measured (± 1mm) and weighed (±0.01 g) prior to dissection and removal of dorsal muscle tissue (ca. 1 cm^3). Tissue was washed in distilled water and dried at 60°C for 48 h and subsequently ground to a fine powder using a mortar and pestle. Ground samples were packed into 5 x 8 mm tin capsules for $\delta^{13}C$ and $\delta^{15}N$ analysis. Lipid extraction or normalisation was not performed as C:N ratios of all fish were within a narrow range (3.3 – 3.4) and as normalisation of tissues with ratios less than 4 (non-fatty) show variances less than 1‰ when compared with untreated values (Gray et al. 2004; McConnaughey and McRoy 1979).

Samples were analysed for stable carbon and nitrogen isotopic composition in the Department of Archaeology at the University of Cape Town. Samples were weighed to an accuracy of 1 microgram on a Sartorius MP2 micro balance and were then combusted in a Flash 2000 organic elemental analyser and gasses passed to a Delta V Plus isotope ratio mass spectrometer (IRMS) via a Conflo IV gas control unit, Thermo Scientific, Bremen, Germany. Results are expressed as per mil (‰) using the standard delta unit notation (δ) defined by the equation: $\delta^{13}C$ or $\delta^{15}N$ (‰) = $[(R_{sample}/R_{standard)} – 1] \times 1000$, where R_{sample} is the ^{13}C:^{12}C or ^{15}N:^{14}N ratio of the sample and $R_{standard}$ is the ratio of the appropriate standard (Vienna Pee Dee Belemnite for carbon and atmospheric N_2 for nitrogen). The precision of measurements was within 0.1‰ for both nitrogen and carbon.

4.3.4. Data analysis

Tests for normality were performed using Shapiro-Wilk and visual observation which indicated that catch data were not normally distributed, even after the appropriate data transformations were performed. Consequently, non-parametric statistical analyses were used to test for differences in fish length between sites and between marked versus recaptured fish. A Mann-Whitney two sample t-test was performed to determine differences in fish length between sites. To determine if recaptures were a subset of the marked population (i.e. no size bias), Mann-Whitney two-sample t-tests were used to compare means and Levene's test was used to compare variance between samples. Physico-chemical measurements were normally distributed and were compared using Student's *t* tests to test for differences between site and sampling year. Results are reported as mean ± standard deviation (SD) for parametric and means with range (min – max) for non-parametric data.

4.3.5. Isotopic niche

Stable isotope ratios were used to assess differences in the trophic interactions of *R. holubi* captured from the two mangrove habitats by assessing the location and width of their isotopic niches. Initially, variations in food source origin and trophic level as a function of ontogeny were tested using linear regression between fish size class and $\delta^{13}C$ and $\delta^{15}N$ to determine the effect of size on isotopic niche space.

Preliminary analysis revealed that smaller juveniles at both study sites were found to occupy a reduced total niche space, samples were therefore split into size groups with small juveniles representing those < 55 mm (TL) and large juveniles representing those > 56 mm. Niche location and width for each group was determined using metrics based on the position and Euclidean distance between isotope data points in bivariate space (Layman et al. 2007a). The location of the centroid, or the bivariate $\delta^{13}C$-$\delta^{15}N$ mean, which represents the average niche position, was compared between groups using non-parametric permutational MANOVA (PERMANOVA (Anderson et al. 2008)). Quantitative comparisons of the amount of variance for each group were made by comparing dispersion between the isotope values for groups. This was achieved by using the test for homogeneity of multivariate dispersions (PERMDISP (Anderson et al. 2008)), a multivariate analogue to Levene's test. The average deviation of isotope points to group centroids was compared for each group where P-values were obtained using permutation of least squares residuals. For both the PERMANOVA and PERMDISP tests, the bivariate isotope data were first normalised, then converted to a

Euclidean distance matrix. For the PERMANOVA test, unrestricted permutation of raw data was used and 9999 permutations were set for both tests.

To compare the isotopic niche widths of the two *R. holubi* size groups at both locations, multivariate ellipse-based metrics, a bivariate equivalent of the standard deviation in univariate space, were constructed following methods by Jackson et al. (2011). The Standard Ellipse Area (SEA) is used to describe the niche width of both community members as well as entire communities and the corrected estimate (SEA_C) is a robust metric of niche space with little bias in respect to varying sample sizes and is not heavily influenced by outliers (Jackson et al. 2011). Further metrics of isotopic niche space were calculated based on methods by Layman et al. (2007a). These included convex hull area (TA), mean nearest neighbour distance (MNND), and mean distance to centroid (CD) (Layman et al. 2007a). All metrics were determined using Stable Isotope Analysis in R (SIAR) package (Parnell et al. 2010) and Stable Isotope Bayesian Ellipses in R (SIBER) package (Jackson et al. 2011), all statistical analysis were performed using R version 3.2.5 (The R foundation for Statistical Computing) with significance for statistical tests set to $\alpha = 0.05$. PERMANOVA and PERMDISP operations were performed using PRIMER & PERMANOVA add-on (Versions 6.1.13 and 1.0.3).

4.4. RESULTS

Summer water temperature differed significantly between sampling events ($t = 7.7$ $P < 0.001$) with warmer conditions in 2015 (22.7 ± 0.9 ºC) than in 2016 (19.6 ± 0.9 ºC) but not between sampling sites ($t = 0.18$, $P = 0.85$). Salinity values were stable and did not differ between sampling events ($t = 1.74$, $P = 0.1$) nor between sites ($t = 1.59$, $P = 0.14$). As sites were located near the estuary mouth salinity levels were near that of marine water (31.7 ± 1.6 PSU). Water turbidity was significantly different between sampling events ($t = 2.28$, $P = 0.03$) and sample sites ($t = 2.38$, $P = 0.03$) with generally clearer conditions at the red mangrove site (2.9 ± 1.8 NTU) compared to the white mangrove site (6.9 ± 5.3 NTU). Dissolved oxygen did not differ either between sampling events or sites (8.9 ± 2.0 mg/L).

4.4.1. Mark-recapture

A total of 746 *R. holubi* were marked and released over the study period (341 and 405 from 2015 and 2016, respectively) with sizes ranging from 27 – 170 mm (TL). Nearly equal numbers of individuals were marked at both locations (378 and 368 at white (WM) and red mangrove (RM) sites, respectively) although effort (measured in number of seine hauls) was higher at WM, with an average of nearly three hauls typically being required to capture the daily target of 50 individuals compared to one at RM.

In total, there were 150 recaptures representing 20.1 % of the total marked population (Table 4.1). Rates of recapture were higher at WM, where 97 individuals were recaptured, compared to RM with 53 recaptures, representing 25.7 and 14.4 %, respectively. The short duration of the study allowed for individuals to be recaptured at most with 4 d at liberty and while this occurred, the majority of recaptures (73%) were made within 2 d from date of tagging.

The size distributions of marked *R. holubi* differed significantly between sites (U = 63 583.5, P = 0.02) with the RM population having a larger average length of 65.2 mm (27 – 170) than the WM population at 62.9 cm (28 – 150). This was consistent with the recaptured population (U = 1125, P < 0.001) where the average length from RM of 71.8 cm (42 – 127) was notably larger than the average of 56.2 cm (37 – 125) from WM (Figure 4.2).

Recaptures at WM appeared to be representative of the marked population, i.e. the two samples came from the same population (W = 19028, P = 0.56) and were homoscedastic (F = 0.62, P = 0.94). However, recaptures from RM had a larger mean, which was significantly different to the marked population (W = 7486, P = 0.006), but were nonetheless homoscedastic (F = 1.4, P = 0.22). Caution is expressed with the results of these statistical tests as sample size between marked and recaptured differed greatly, a factor leading to unreliability in many statistical tests. Plots however tend to visually support the above findings (Figures 4.2 and 4.3).

Table 4.1: Location, number of marked and recaptured with number of days at liberty for *Rhabdosargus holubi* at the Mngazana Estuary, South Africa.

	White mangrove	**Red mangrove**	**Total**
No. marked	378	368	**746**
Mean (range) mm	62.9 (28 – 150)	65.2 (27 - 170)	
Days since marked			
1	49	26	
2	19	15	
3	20	10	
4	9	2	
No. recaptured	97	53	**150**
Mean (range) mm	56.2 (37 – 125)	71.8 (42 – 127)	
Percent recaptured	25.7	14.4	**20**

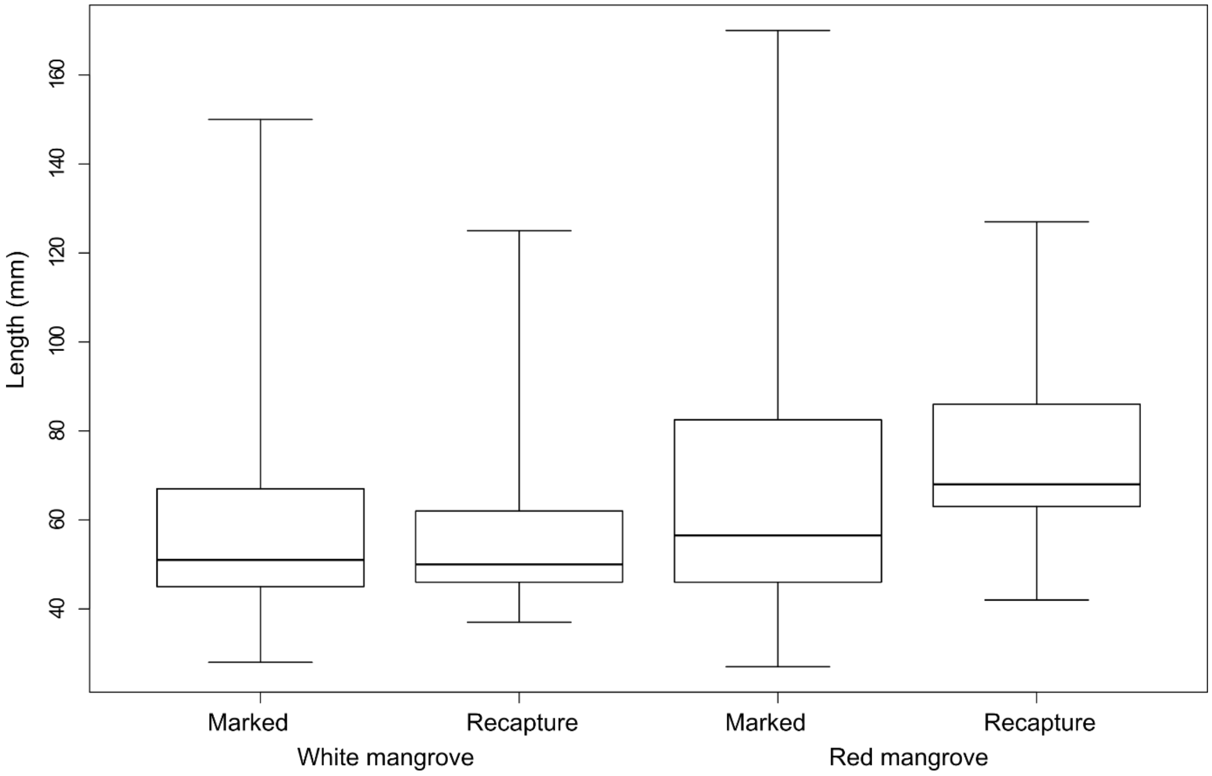

Figure 4.2: Length distributions for marked and recaptured *R. holubi* at the white and red mangrove sites. Boxes represent 25 and 75% interquartile range with bar representing median, whiskers show minimum and maximum range.

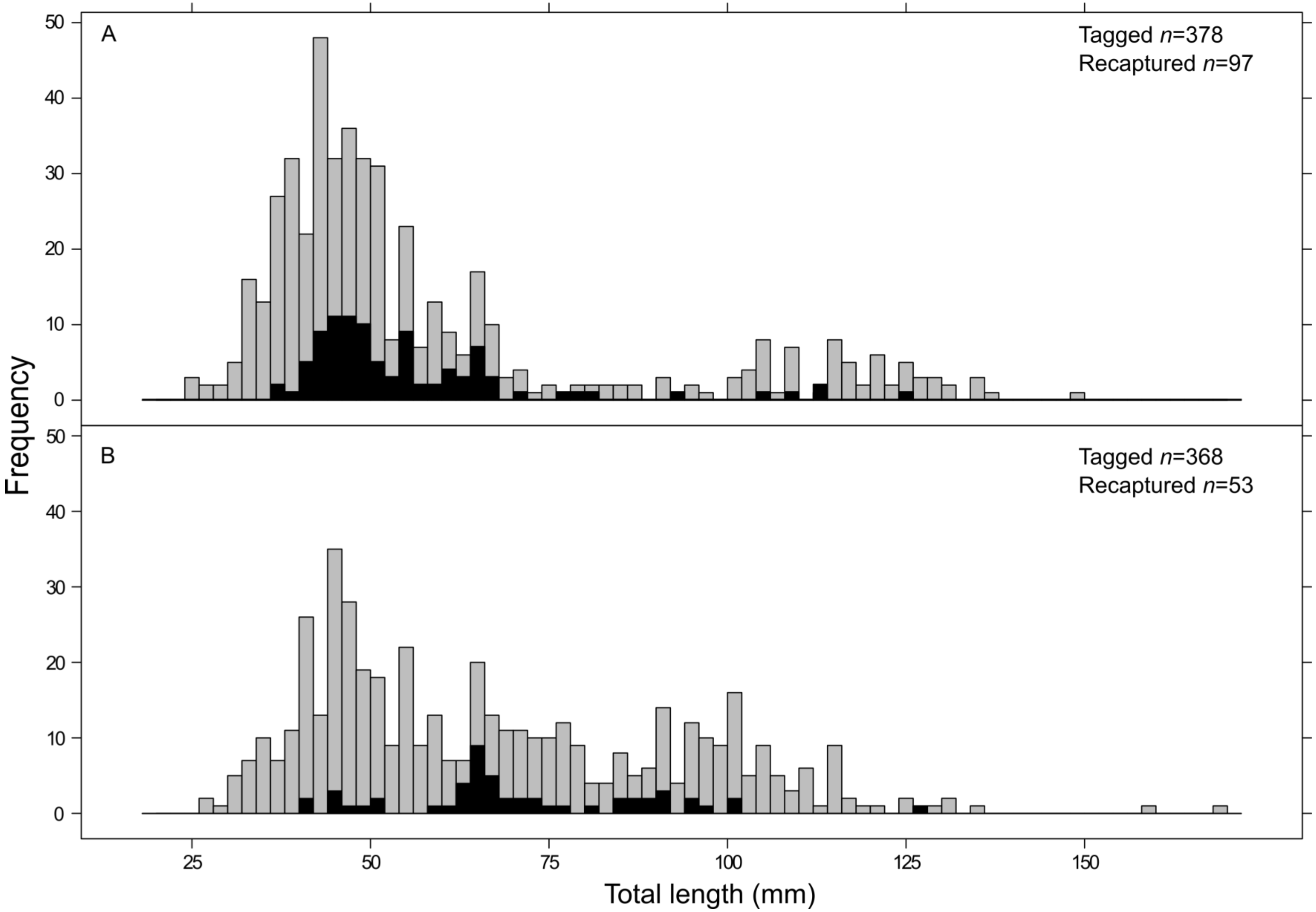

Figure 4.3: Size frequency histogram for marked (grey) and recaptured (black) *R. holubi* at Mngazana Estuary for (A) white mangrove, and (B) red mangrove populations.

4.4.2. Stable isotopes

The $\delta^{15}N$ values of *R. holubi* increased along a gradient of fish size which, although significant ($F = 13.1$, $P < 0.001$, $N = 84$), was nonlinear ($r^2 = 0.14$) due to wide variability of nitrogen values throughout the range of fish sizes (Figure 4.4), even though the total recorded $\delta^{15}N$ was within a narrow range (uncorrected values ranged from 11.3 – 13.7 ‰). When the two habitats were analysed separately, length and $\delta^{15}N$ for the WM sample exhibited the same trend reported above ($r^2 = 0.13$, $F = 9.7$, $P = 0.004$, $N = 40$) but the RM sample, conversely, showed no linear correlation ($r^2 = 0.009$, $P = 0.5$, $N = 44$). The $\delta^{13}C$ values did not show a significant linear correlation along size gradients when analysed collectively or by habitat. The $\delta^{15}N$ and $\delta^{13}C$ values for WM and RM samples varied significantly ($t = 6.93$, $P < 0.0001$ and $t = 3.81$, $P < 0.001$, d.f. = 79 for $\delta^{15}N$ and $\delta^{13}C$ respectively). The WM isotopic niche was represented by a tight clustering of individual isotope values on the $\delta^{15}N$-$\delta^{13}C$ biplot with constrained ranges (1.27 and 1.85‰, respectively) relative to the more liberal ranges from the RM sample (2.36 and 6.59‰, respectively). Mean $\delta^{15}N$ and $\delta^{13}C$ for the RM sample was enriched relative to the WM sample.

4.4.3. Isotopic niche

Comparisons of $\delta^{15}N$-$\delta^{13}C$ centroids revealed that the isotopic niches from the two habitats were separate ($F = 30.6$, $P < 0.001$). Pair-wise tests revealed that centroids between small juveniles (<55 mm) and large juveniles (>55 mm) from the same habitats were more similar than between habitats, where either small or large groups were consistently and significantly different (all pair-wise tests: $P < 0.05$ (Table 4.2)).

Deviations from centroids, either within or between habitats, were all significantly different (all pair-wise tests: $P < 0.05$ (Table 4.2)) indicating that neither small nor large groups occupied similar niche widths. Furthermore, the mean bivariate dispersions were larger for

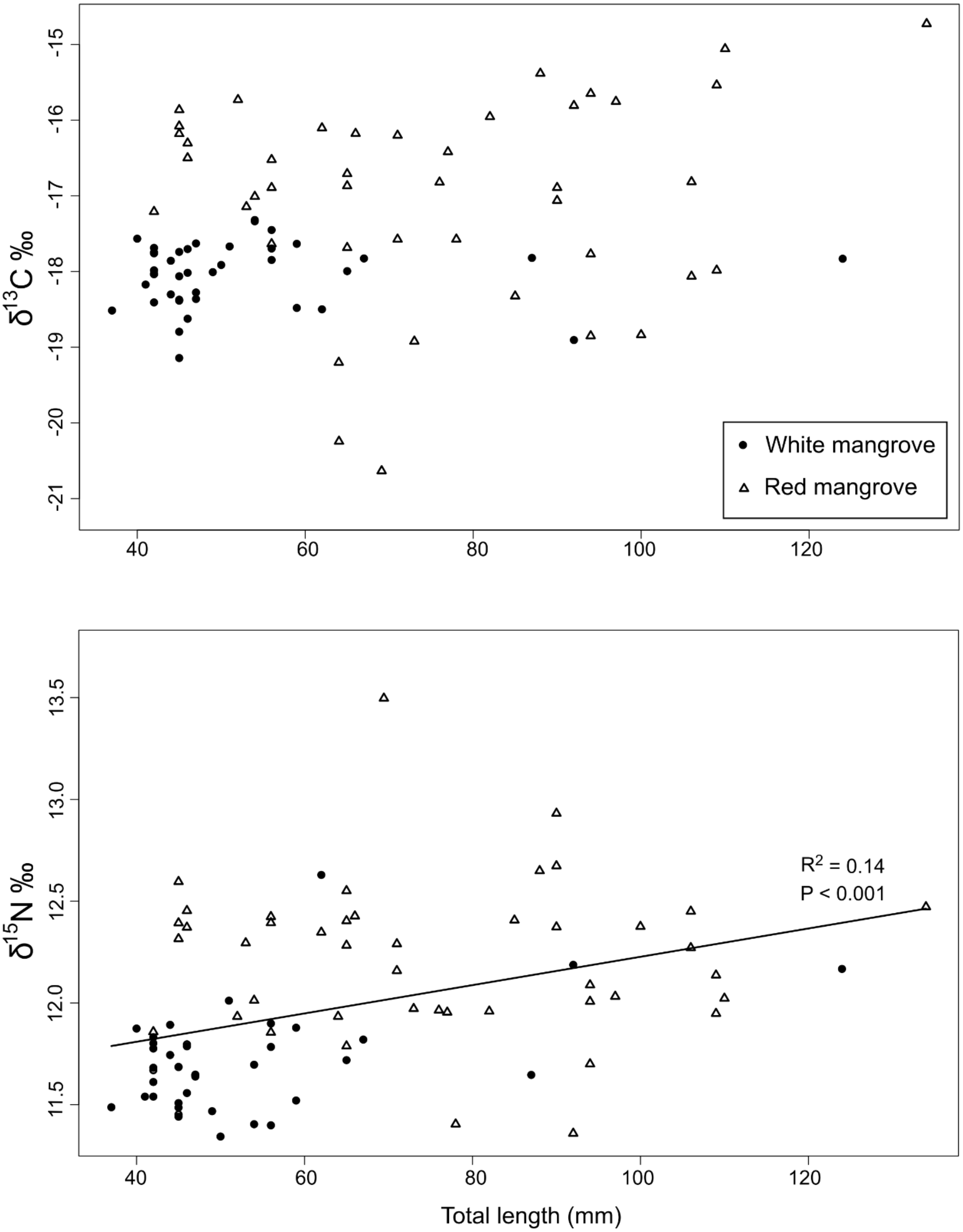

Figure 4.4: The δ^{13}C and δ^{15}N values measured as a function of *R. holubi* size from the two study sites in the Mngazana Estuary (trend line and R^2 only shown when significant).

both small and large juveniles from the RM habitat indicating broader isotopic niche area for the groups from this habitat.

Mean estimates of standard ellipse area (SEA_B) showed that the isotopic space of even small *R. holubi* juveniles at RM was greater in area than the large juveniles at WM (Table 4.3 and Figure 4.5). Bayesian statistics indicated a high probability that the SEA_B of the total RM sample is larger than that of the total WM sample (Probability = 0.008 at 10 000 iterations). Overlap among samples was predominantly between small and large juveniles from the same habitat with little overlap for large and none for small juveniles between sites (Figure 4.6). The calculated Layman metrics (Layman et al. 2007a) (Table 4.3), which are more sensitive to sample size, generally showed a similar pattern of results for distance to centroid (CD) as those found using PERMDISP, as well as total convex hull area (TA) to the SEA.

Table 4.2: Comparisons of (a) dissimilarity in terms of centroid locations and (b) multivariate dispersions between sites and size groups sampled at the Mngazana estuary with subsequent pair-wise tests between sites for size groups.

(a) PERMANOVA	Factor	df	SS	MS	F	P
	Site	1	45.11	45.11	30.60	< 0.001
	Groups	3	49.86	16.62	11.45	< 0.001
Pair-wise					*T*	*P*
WM - RM	Small	37			9.07	< 0.001
	Large	43			2.11	0.01
(b) PERMDISP	Factor	df			F	P
	Site	1			22.62	< 0.001
	Groups	3			13.59	< 0.001
Pair-wise					*T*	*P*
WM - RM	Small	37			2.90	< 0.05
	Large	43			2.70	< 0.05

Table 4.3: Summary of isotopic niche metrics for small (< 55 mm) and large (>55 mm) juvenile *R. holubi* at sites sampled at Mngazana Estuary. δ^{13}C range (CR) giving an estimate of the diversity of basal resources, total area of convex hull (TA) encompassing data points giving an indication of niche width, mean distance to centroid (CD) providing information on niche width and individual spacing, mean nearest neighbour distance (NND) providing information on density and clustering, and the core isotopic niche width is represented by the Bayesian estimate of standard ellipse area (SEA$_B$) with 95% confidence intervals.

Group	n	Mean(Range) mm	CR	TA	CD	NND	SEA$_B$
WM Small	27	44.4 (37-51)	1.59	0.62	0.36	0.11	0.20 (0.13 – 0.28)
WM Large	13	71 (54-124)	1.57	1.55	0.66	0.37	0.45 (0.23 – 0.71)
RM Small	12	49.6 (42-56)	1.84	0.86	0.55	0.23	0.47 (0.23 – 0.76)
RM Large	32	86.3 (64-134)	6.59	8.45	1.39	0.35	2.16 (1.47 – 2.92)

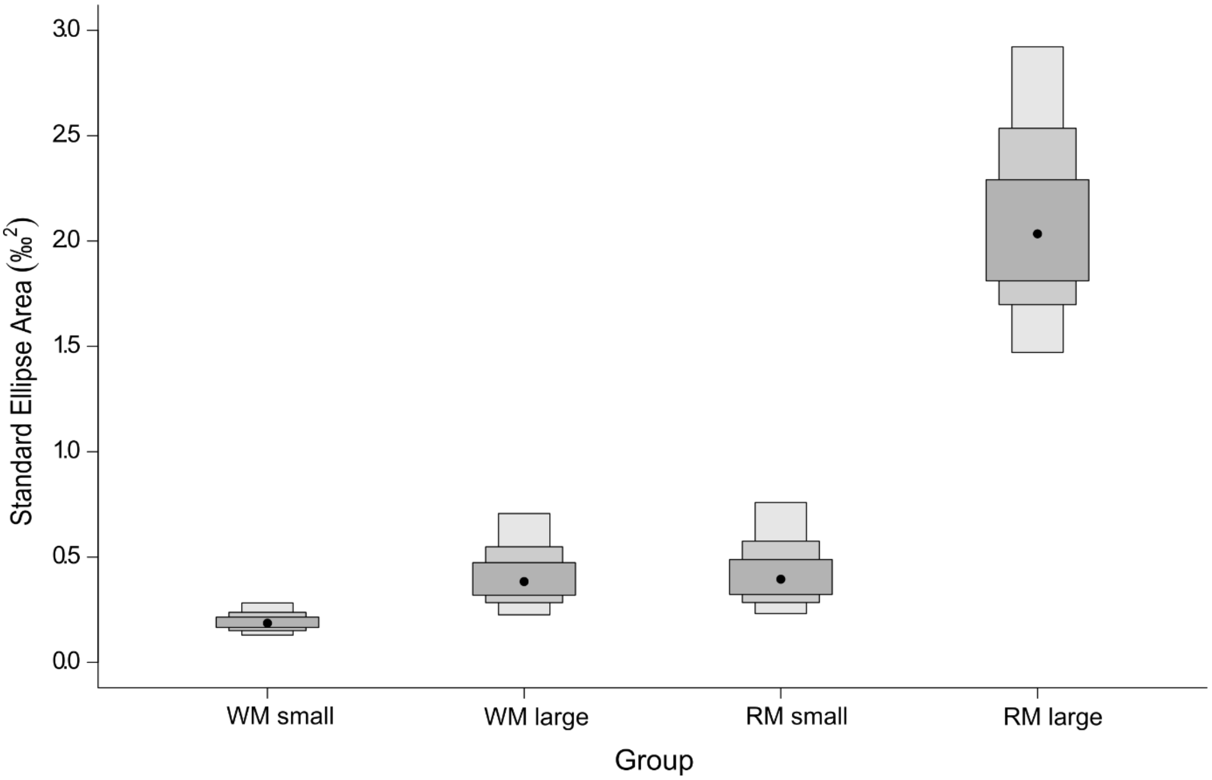

Figure 4.5: Estimated posterior distributions of small and large juveniles of *R. holubi* from white and red mangrove habitats showing 50%, 75% and 95% credible intervals.

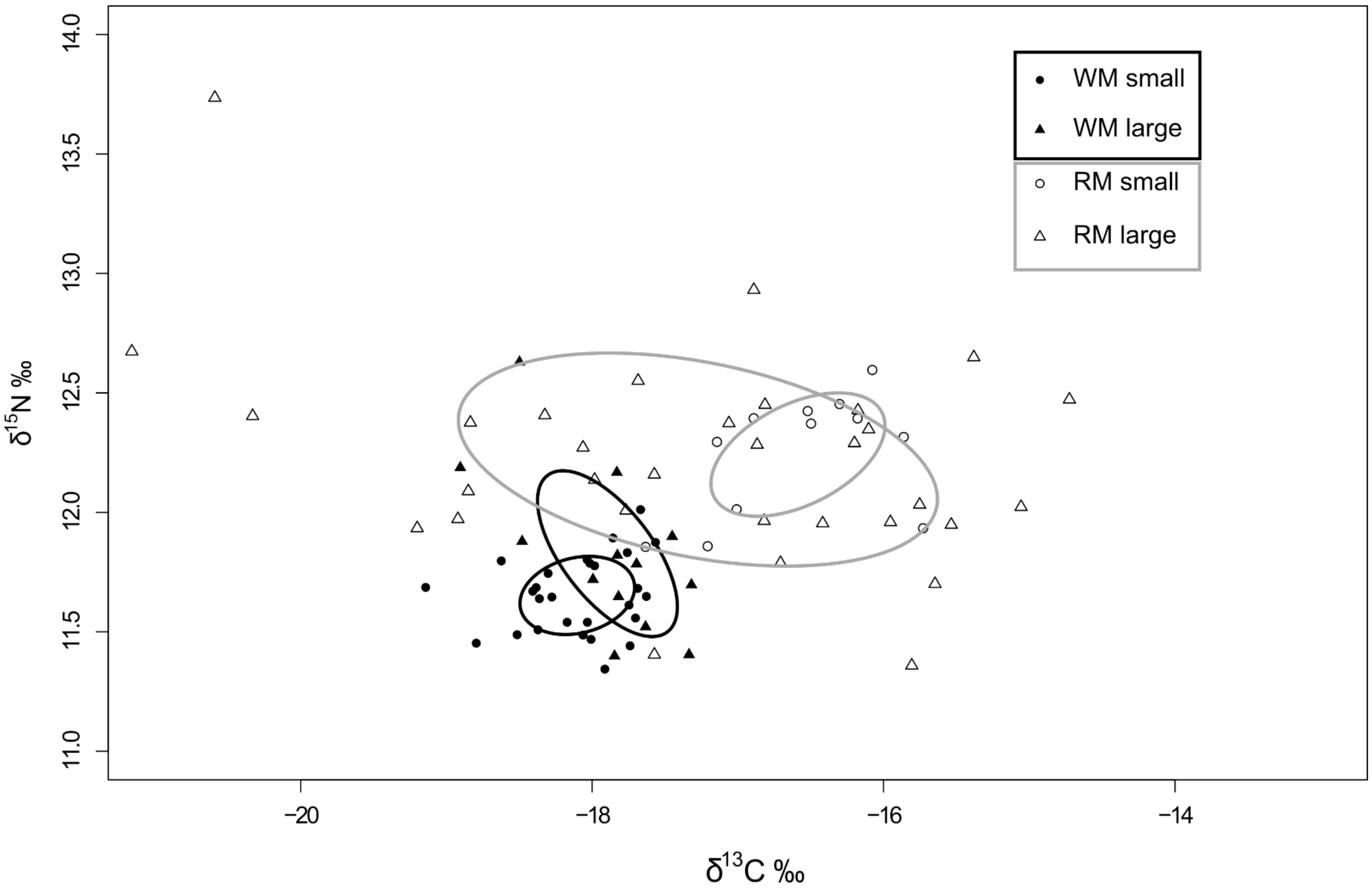

Figure 4.6: Isotopic niches of juvenile *R. holubi* at white and red mangrove sites represented by standard ellipse areas (SEAC; 40%). White mangrove small (n = 27) and large (n = 13), red mangrove small (n = 12) and large (n = 32).

4.5. DISCUSSION

Mark-recapture of the marine-estuarine dependent *Rhabdosargus holubi* indicates a considerable level of short-term site residency with comparatively high rates of recapture irrespective of mangrove root type in the lower Mngazana Estuary. Similar short-term studies (on the order of days) have had much lower recapture rates in similar environments. A recapture rate of 1.3 % (four recaptures from 300 tagged) was recorded for juvenile red drum *Sciaenops ocellatus* within three days of tagging in estuarine sea grass beds using VIE (Bushon et al. 2007). Pinfish *Lagodon rhomboides* in a west Atlantic estuary study yielded a total recapture rate of 18.3 % (437 from 2 297 tagged) over 23 weeks, however, 9.2 % of total recaptures (226 individuals) were made on fish within one week of tagging with the authors concluding that pinfish exhibit strong site fidelity (Potthoff and Allen 2003). Skinner et al. (2005) evaluated residency of the mummichog *Fundulus heteroclitus* in a Canadian estuary with a VIE mark-recapture study. Repeated mark-recapture took place over four months where 4 123 individuals were marked at four creeks with recapture sampling taking place biweekly yielding a total recapture rate of 12.7% (524 individuals). It was concluded that mummichogs, like pinfish, exhibit a high degree of site residency (Skinner et al. 2005).

In the present study, 150 individuals were recaptured from a marked population of 746 *Rhabdosargus holubi*, representing a total recapture rate of 20.1 %. Recapture rates were higher (26 %) at the comparatively more homogenous white mangrove site along the main channel. This shore region is made up of a monospecific mangrove grove where the shallow pneumatophore banks are bounded by the deep main channel of the Mngazana Estuary. The deep channel likely forms a migration barrier for juveniles but the continuous shallow banks are expected to provide a corridor between adjacent habitats like the shallow creek of the red mangrove site. The slightly lower recapture rate at the red mangrove creek may be attributed to the fringing eelgrass beds which not only provide a valuable nursery habitat for *R. holubi* (Whitfield et al. 1989) but also provide a connective route between opposite banks of the creek.

A key assumption for tag-and-recapture studies is that past captures will not have an influence on the animal's probability of survival or recapture (Crosbie and Manly 1985). Evidence of this would be displayed in a bias of recapture size distributions where smaller individuals may be faced with higher rates of mortality and larger individuals may have greater dispersal. It was found that recaptured populations fell within the range of the tagged population indicating little variability in movement for both small and large juvenile size classes.

Stable isotope ratios in *R. holubi* subpopulations captured at white and red mangrove habitats showed a significant difference between sites and size groups analysed. The small juveniles (< 55 mm) in particular showed no overlap in the standard ellipse areas where separation along the $\delta^{13}C$ axis indicates assimilation of a separate set of sources and separation along the $\delta^{15}N$ axis indicates assimilation of sources from a potentially higher trophic level. Small juveniles from the white mangrove site had relatively C depleted signatures when compared to those from the red mangrove site indicative of a greater contribution of mangrove derived sources. As isotopic signatures of mangroves are consistently found to be depleted relative to the enriched values of eelgrass/seagrass (Vaslet et al. 2011; Mbande et al. 2004; Cocheret De La Morinière et al. 2003) it is reasonable to predict that these differences in isotopic niche space or SEA are due to the separation of habitats and the assimilation of prey or other resources from those habitats.

Larger juveniles (> 55 mm) exhibited marginal overlap in their SEA but those from the white mangrove site essentially formed a subset of the much broader range in $\delta^{15}N$ (2.4 ‰) – $\delta^{13}C$ (6.4 ‰) of those from the red mangrove site. This greater range combined with the average enriched values indicate assimilation from more diverse basal sources, likely a result from the combination of mangrove and eelgrass which are characteristic of this site. Conversely, the clustered, narrow range in $\delta^{15}N$ (1.3 ‰) and $\delta^{13}C$ (1.8 ‰) of the white mangrove population suggest the assimilation of a narrower range of prey which is likely from a limited geographical range (Bearhop et al. 2004). Despite the presence of eelgrass nearby (within

300 m), stable isotopes of white mangrove individuals fell within a narrow range indicative of mangrove carbon sources which, by using muscle tissue, indicates that for at least a month prior, no assimilation of eelgrass based resources has taken place.

Layman et al. (2007b) explored the response of $\delta^{15}N$ – $\delta^{13}C$ niche width for grey snapper *Lutjanus griseus,* a top predator in tropical mangrove systems, in habitats ranging in their degree of anthropogenic fragmentation. Grey snapper populations from fragmented habitats consistently and significantly had smaller niche width due to contraction along both axes (Layman et al. 2007b). This was attributed to the homogenisation of these habitats where pathways of energy flow are constrained to originate from a reduced basal resource pool (Layman et al. 2007b). Quevedo et al. (2009) similarly showed that niche reduction can occur at the intrapopulation level associated with differential habitat use. Eurasian perch *Perca fluviatilis* are found to separately occupy pelagic and littoral habitats within lakes. The pelagic environment was found to be more homogenous with a less diverse prey community, subsequently the pelagic subpopulation had a smaller isotopic niche and showed smaller distances to the isotopic centroid (i.e. were more clustered), indicating lower trophic diversity than the littoral population (Quevedo et al. 2009). The mechanism responsible for the separation of isotopic niches for perch was found to be similar to that for snapper, namely lower prey diversity (Quevedo et al. 2009).

In the present study, habitats along the main channel were comparatively homogenous where a single basal resource, the white mangrove, appears to form the basis of the resource pool. The red mangrove creeks, alternatively, offer a more heterogeneous habitat where epiphytes and epifauna associated with mangroves and eelgrass can be found, this ultimately results in a wider feeding niche for fishes occupying this area. Residency within the two habitats led to a marked difference in the isotopic niche of *R. holubi*. Carassou et al. (2016) similarly found overlapping $\delta^{13}C$ values for *R. holubi* juveniles between the ontogenetic movements along the river-estuarine-ocean ontogenetic stages, indicating a degree of residency during different stages of its life cycle along this environmental continuum. Results from our study indicate that, apart from major ontogenetic movements between ocean and estuary, *R. holubi* establish high rates of residency for sites soon after recruitment into estuaries and that not only does this species exhibit residency within the estuary as a whole but may be resident to areas in the range of meters during the juvenile phase despite variations in refuge and food availability amongst habitats.

Estuaries and their habitats, such as saltmarshes, eelgrass beds and mangrove forests, are areas of high productivity and are valuable nursery areas for juvenile fishes in what can be viewed as a spatially patchy environment (Beck et al. 2001; Whitfield 1999). The actual value of essential fish habitat or habitat quality is, however, difficult to estimate with the premise that some habitats contribute disproportionately to adult populations (Beck et al. 2001; Able 1999). If we assess the two habitats from this study on a unit-area basis, as suggested by Beck et al. (2001), it appears superficially that the shallow creek of the red mangroves provides a comparatively more valuable habitat for *R. holubi* as it offers greater feeding and refuge opportunities. Actual patch quality however can only be estimated with rates of derived fitness despite densities, based on catch per unit effort, being consistently higher at the red mangrove habitat.

In spatially heterogeneous environments, such as estuaries, the distributions of many species are often found to be non-random as they show patch preference or are otherwise said to exhibit a coarse-grained response (Weins 1976). Indeed, an optimal adaptive response would be for individuals to maximise personal fitness by selecting the most advantageous habitats. Habitat selectivity responses have been found to occur in tropical reef fishes which have coastal juvenile nursery phases where mangrove or seagrass habitats are actively sought out (Huijbers et al. 2012; Nakamura et al. 2009). Temperate estuaries are however, by nature, highly unpredictable environments (Whitfield 1994b). The plasticity or ability of a species to occupy varying habitats in these systems may therefore be an evolutionary stable strategy where the ‘spreading the risk’ strategy in a heterogeneous environment may contribute to population stability (Reddingius and Den Boer 1970).

Conversely, high densities of fishes within optimal habitats may lead to a decline in patch quality due to intraspecific competition. Ideal free distribution theory suggests that this decline in patch quality leads to a broader range of habitats becoming equally suitable (Weins 1976). However, fulfilment of the assumptions for an ideal free or despotic distribution in natural systems are unlikely to occur as real time knowledge of patch quality would be costly and equivocal fitness amongst habitats is questionable.

Density-dependent factors could be responsible for the distributions observed in the current study but it is more plausible that *R. holubi* is a eurytopic species able to adapt to a wide range of seemingly less valuable habitats. Levin et al. (1997) found that pinfish in western Atlantic lagoons showed a high preference for seagrass beds, where they were present in high densities, but sand plains appeared to present a viable alternative habitat despite growth rates being higher in seagrass habitats. The authors concluded that size selective predation and habitat differences in growth rate were key to higher recruitment in vegetated habitats (Levin et al. 1997).

In conclusion, by using mark-recapture and stable isotope analysis the study provides evidence for both short- and long-term site residency of *Rhabdosargus holubi* in two dissimilar mangrove root habitats. Residency within a shallow creek associated with red mangrove and eelgrass led to a wider isotopic niche than those from the comparatively more homogenous white mangrove habitat. This indicates assimilation of a broader set of resources from the more heterogeneous habitat where individuals also typically fed at a higher trophic level. It appears that, despite major ontogenetic movements, this abundant and highly successful marine estuarine dependent species shows high degrees of residency in certain habitats once settled. The distribution of this species within estuaries and in habitats which vary in quality is likely a result of an interaction between recruitment variability, intraspecific competition and predation. Knowledge of the extent to which fish move and utilise habitats is critical information for fish ecology, effective management and conservation (Gillanders 2009). Movements of fishes are said to be in response to a complex mix of abiotic (salinity, temperature) and biotic (competition, predation) conditions found within estuaries (Rountree and Able 2007) but for a species where juveniles are highly tolerant of a wide range of abiotic variables, the least costly response may be to not move at all.

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CHAPTER 5

SYNTHESIS AND CONCLUSIONS

In a review of research on fishes in mangroves over the last 50 years, Faunce and Serafy (2006) covered 111 papers with only one originating from a temperate climate, namely that of Bell et al. (1984) from south-eastern Australia. More recent studies have been performed in temperate Australia and New Zealand and while some of these have supported findings of higher juvenile abundance and richness in mangrove habitats (Hindell and Jenkins 2004), the majority appear to confirm that warm temperate mangrove nurseries are no more important than other adjacent nursery habitats typical of warm temperate estuaries (Payne and Gillanders 2009; Bloomfield and Gillanders 2005; Smith and Hindell 2005; Clynick and Chapman 2002). Results from juvenile distributions within warm temperate South African estuaries (Chapter 2) similarly showed that fish communities were not dissimilar amongst habitats, provided the underlying sediment was the same. Abundance and species richness showed no significant variation among eelgrass, mangrove and even non-vegetated mudbanks both within and among sampled estuaries, corresponding to results by Payne and Gillanders (2009) from southern Australia. No species were found to be unique to temperate mangrove habitats and species composition between mangrove, eelgrass and mudbanks were near identical. Faunce and Layman (2009) suggested that tidal fluctuations may be responsible for such observations where fishes are forced to utilise littoral or adjacent mudflats twice daily during ebb tides. More recently, Edworthy and Strydom (2016) suggest plasticity of use of habitats by juveniles in warm temperate estuaries.

Using a seascape nursery perspective, Nagelkerken et al. (2001) found that tropical Caribbean embayment’s with mangrove habitats had more species throughout and at higher densities than alternate bays without mangrove habitats. Similarly, Jelbart et al. (2007) in temperate south Australia, found that fish assemblages in seagrass beds were influenced by the proximity of mangrove forests. Both authors concluded that connectivity amongst a diverse assemblage of habitats was key in sustaining greater densities and species richness. As such, it was anticipated that warm temperate mangrove systems along the south-eastern coastline of South Africa would harbour higher densities from a more diverse fish assemblage. However, juvenile fish communities from the mangrove estuaries of Nahoon and Xhora were not dissimilar to those from Gonubie and Qora were mangroves are absent.

Early stage fish communities, which included larval and early juveniles, similarly showed no variation between estuaries with and without mangroves. Mumby et al. (2004), in a Caribbean study, found that the biomass of several species on coral reefs was more than doubled when mangroves were functionally connected to adult habitats. They concluded that mangroves serve important nursery and intermediary habitats maintaining the functioning, fisheries, biodiversity and resilience of Caribbean coral reefs (Mumby 2006; Mumby et al. 2004). This functional connectivity among adult and juvenile habitats in turn results in greater larval supply to nearby estuarine and mangrove habitats (Nagelkerken et al. 2008). Marine-spawned early stage fishes captured in the current study were no more abundant within mangrove estuaries than those without. Dissimilarity between fish communities was rather latitudinal with northern estuaries containing a richer assemblage with higher densities. While some of the dissimilarity may be attributed to a latitudinal shift in species richness, Generalised Additive Models suggested rather that freshwater input appears to provide an overarching influence on warm temperate fish communities with temperature and season influencing the abundance of many fish species. These findings being supported by results from many other studies of fish assemblages in South African estuaries (Strydom 2015; Whitfield 2005; Whitfield 1999; Whitfield 1994).

In tropical mangrove fish studies, high densities within these habitats are typically attributed to a few mangrove-dependent species commonly being representatives of the families Haemuliade and Lutjanidae, the grunts and snappers, respectively (Nagelkerken and Faunce 2008; Nakamura et al. 2008; Mumby et al. 2004). These marine-spawned species utilise these habitats as either nurseries or as intermediate feeding habitats before migration to offshore adult habitats (Faunce and Serafy 2008). Marine-spawned estuary dependent species are similarly common in South African estuaries, making up the majority of species present, with representatives of Haemulidae also represented in warm temperate systems (Whitfield 1998; Whitfield 1990). There however does not appear to be any mangrove-dependent species represented in warm temperate South African estuaries nor southern Australia or New Zealand, according to Morrisey et al. (2010). Commonly occurring marine estuary-dependent species such as *Rhabdosargus holubi, Sarpa salpa* and *Monodactylus falciformis,* appear to rather be macrophyte-dependent occurring in higher densities in any vegetated sites (e.g. *Zostera, Spartina, Potamatopon*) compared to bare substrate (Whitfield 1999). The need for species to be flexible in warm temperate estuarine systems which are unpredictable, often facing stochastic events and wide ranging seasonal variability (Whitfield 1990), may be more important than for those inhabiting comparatively stable tropical systems. Especially given that warm temperate mangrove habitats are found to offer no more protection from predation than alternate habitats (Smith and Hindell 2005) and that they are in fact found to be regions of comparatively low productivity (Alongi 2009; Clarke 1994).

Despite some general associations, plasticity in habitat use may be more common than anticipated and has been shown for a number of species including the engraulid *Lycengraulis grossidens* (Mai et al. 2014), sciaenid *Sciaenops occellatus* (Bacheler et al. 2012) and sparid *Rhabdosargus holubi* (Becker et al. 2010) in estuarine environments. Distributions of the common estuarine dependent sparid *R. holubi* confirmed a high degree of plasticity in habitat use. Densities and size classes from two dissimilar habitats were similar with the species showing a high degree of habitat residency for both habitats as determined by short-term, VIE tagging and long-term, isotope analysis. Isotope analysis revealed that individuals occurring in a more heterogenous habitat had a wider feeding niche than those from a more homogenous habitat, which could potentially lead to slower growth rates. Early settlement and little movement amongst habitats may however prove more beneficial to survival than increased growth rates. The ability of *R. holubi* to populate various habitats equally well may be the reason as to why they are so successful or abundant in warm temperate South African estuaries.

Interest in the role of mangroves as nursery habitats has led to a wealth of studies on the topic with varying results and conflicting conclusions. Faunce and Layman (2009) identify common sources of variability among studies often leading to conflicting results including spatial, temporal and species variation among studies. Spatial variation in results has been found among ocean systems with western Atlantic findings typically enforcing the view of mangrove nurseries being important for fishes while those from the Indo-Pacific (which includes East Africa) have challenged these views (Faunce and Layman 2009). Differences in habitat configuration may be responsible for these observations as Western Atlantic mangroves occur widely in low-relief regions and studies have focussed on areas (e.g. Florida and Caribbean) where low tidal fluctuations often result in mangrove habitats being permanently inundated (Faunce and Serafy 2006). Another factor leading to contradictory findings is due to the level of observation which may either be at the species or community level. Species based studies are typically focussed on those species which are mangrove-dependent and as such their value, in terms of density and growth within these habitats, typically enforce the value of these environments (Nakamura et al. 2008; Mumby et al. 2004). Community based studies however often find little variability in species richness and density among habitats. Furthermore, there is much intraspecific variation which is seldom considered in studies on nursery value where species may be able to utilise alternate habitats equally well. Layman et al. (2007) for example showed that the habitat use and foraging behaviour of a generalist species increased in more heterogeneous environments. Results from the distribution and habitat use of *Rhabdosargus holubi* appear to confirm this prediction where along much of their warm temperate distribution mangroves are not present, however when these habitats are accessible they are also utilised seemingly quite successfully by *R. holubi*.

In conclusion, results from early stage and juvenile fish communities revealed no significant dependence on mangrove habitats with these proposed valuable nursery habitats providing little effect on fish communities in terms of diversity and abundance in warm temperate South African estuaries. This is not to say that their value as a nursery is absent but rather equivocal to other shallow estuarine habitats within the same ecosystem. An absence of any mangrove-dependent species is likely attributed to two major factors. Firstly, tidal variation within South African estuaries means that these habitats are not permanently inhabitable. Secondly, the unpredictable nature of South African estuaries with wide seasonal variability favours generalist species with wide niche overlap (Whitfield 1990). Within estuary densities of fishes were likely not enhanced by the presence of mangroves because these primary producers are found to conversely have low production rates in temperate climates contributing little to overall productivity within systems. Furthermore, the structural component of these habitats and refuge provision have been found to be similar amongst alternate vegetated habitats and unvegetated shallow water habitats (Smith and Hindell 2005). Finally, while habitat variability has been found to impact species diversity (Strydom 2015; Whitfield 1999) the overarching influence of temporal freshwater input with associated affects in salinity gradient, turbidity and importantly productivity exert a far stronger influence on warm temperate fish communities potentially overshadowing any influence derived by the presence of mangrove habitats.

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