Assessing estuarine nursery habitats for Cape Stumpnose (*Rhabdosargus holubi*), (Pisces: Sparidae) in a warm-temperate estuary in the Eastern Cape, South Africa.

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ABSTRACT

The nursery role hypothesis provides an approach for assessing the nursery function of habitat types within estuaries. This study attempted to assess the nursery value of the dominant estuarine habitats in the Bushmans Estuary for *Rhabdosargus holubi* (Steindachner 1881) by analysing habitat complexity, relative abundance and behaviour of *R. holubi* and using stomach contents analysis and dietary diatom composition as indicative of feeding habitat.

Structural habitat complexity was assessed in *Zostera capensis* (Setchell) seagrass and *Spartina maritima* (Curtis Fernald) salt marsh by sampling above-ground stem density and length, and total cover per unit area (C_t/A_t). Dimensionless habitat complexity indices such as the interstitial spatial index (ISI) at three magnifications and fractal geometry at two magnifications were used to further analyse habitat complexity. Above-ground biomass ($P<0.05$) in each season and canopy height ($P<0.001$) were significantly higher in salt marsh than in seagrass whilst stem density was significantly higher in seagrass than in salt marsh in each season ($P<0.001$). Each dimensionless index indicated that complexity is notably higher in seagrass than in the salt marsh. Using dimensionless indices that analyse complexity at different spatial scales provided a better analysis of habitat complexity than canopy height and biomass as it allowed for direct comparisons between habitat types.

Underwater video cameras were deployed in seagrass, salt marsh and sand flat habitats to assess the relative abundance and behaviour of *R. holubi*. The relative abundance of *R. holubi* was significantly higher in seagrass than salt marsh and sand flats, whilst the behaviour of *R. holubi* indicated a high degree of habitat use in structured habitats and a low degree of habitat use in unstructured sand flat habitats. This indicated that not only are juvenile *R. holubi* a vegetation-associated species, but also a species that prefers seagrass to salt marsh.

Thirty *R. holubi* individuals were caught in each season, in each of the following areas: the sand flats, mud flats, salt marsh and seagrass. The percent volume, frequency of occurrence and relative importance of general dietary items were identified to family while diatoms in the stomach contents of *R. holubi* and in the *Z. capensis* habitat were identified to genus. The general diet and diatom composition revealed that *R. holubi* is a generalist, opportunistic feeder that feeds in all of the main habitats and hence does not rely on any specific environment for food. The diatoms consumed by *R. holubi* indicated that *R. holubi* feeds on floating filamentous algae and *Z. capensis* leaves in the water column.

As seagrass was not found to be a more important feeding habitat than salt marsh, sand flats and mud flats, the high abundance of and slow meandering behaviour exhibited by *R. holubi* in seagrass indicate that it uses this habitat for protection from predators due to the complexity of the habitat. Secondly, the seagrass beds may be an important feeding habitat in autumn and winter. Results from this study suggest that determining the degree of complexity of various habitats combined with habitat use of a species, by assessing density or abundance and behaviour, is a valuable approach towards understanding the relative nursery value of estuarine habitats.

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CHAPTER ONE

GENERAL INTRODUCTION

Worldwide, the most valuable ecological function of estuaries is their capacity to act as nursery areas for many species of fish and invertebrates (Elliot and Hemingway 2002). Nurseries are areas inhabited by the juveniles of a species, with the adults living in separate habitats (Deegan 1993). Estuaries are generally sheltered, shallow environments that provide protection for juvenile fish species from wave action, strong currents, and a range of predators that inhabit exposed coastlines, whilst also providing large areas for foraging energy rich foods (Orth et al. 1984, Wallace et al. 1984). The coastline of southern Africa is highly exposed to strong currents, heavy wave action and an abundance of predators (Wasserman and Strydom 2011). This differs from other coastlines around the world such as Australia, where sheltered coastal bays function as nursery areas for many commercially and recreationally important fish species (Lenanton 1982, Hampton and Griffiths 2007). Hence, due to the lack of suitable nursery areas along its coastline, estuaries in South Africa are critical nursery areas.

Whilst 1 500 fish species occur on the continental shelf of southern Africa, a relatively small proportion of species are associated with estuaries (Wallace et al. 1984, Whitfield 1999). The degree of dependency of the fish species on estuaries varies markedly (Whitfield 1998). Of the 155 fish species that are associated with South African estuaries, 50% are known to have a strong association with estuaries and 32 (21%) are wholly dependent on estuaries (Whitfield 1999). South African estuaries also support euryhaline marine fish species that utilize estuaries during the juvenile phase of their life cycle (Whitfield 1998). This category includes a sub-category of fish species that are totally dependent on estuaries for the nursery habitats they provide. This sub-category constitutes 11 (7.1%) of the fish species that use South African estuaries (Whitfield 1998).

South Africa has approximately 300 functioning estuaries along its coastline, making up 90 844 ha of the most productive habitats in South Africa (Adams et al. 2012). South African estuaries are broadly categorized into five estuarine systems, namely estuarine bays, permanently open estuaries, river mouths, estuarine lakes and temporarily closed estuaries (Whitfield 1992). Thirty-seven (12.8%) of the estuaries in South Africa have permanent tidal inlets from the sea (Reddering and Rust 1990), with the open-mouth phase largely determined by catchment size (Whitfield 1999). The mouth phase of an estuary plays a primary role in determining fish species diversity; permanently open estuaries have greater diversity, in part due to greater connectivity to the sea (Whitfield 1999). Marine migrants are known to constitute a low percentage of the ichthyofaunal assemblage in temporarily closed estuaries (<1%) but have been found to occur in relatively equal abundances in permanently open estuaries (Bennett 1989). Permanently open estuaries function as critical nursery areas (Whitfield 1998). Habitats in permanently open estuaries provide a predictable area of nursery habitat that is continuously available for use by estuarine dependent marine fish species in the juvenile life-cycle phase. However, less than 50 southern African estuaries are permanently open to the sea and these are the most sensitive to reductions of freshwater inputs (Whitfield 2005).

The habitat types found within estuaries are not equally important to juvenile fish species as nursery areas, as different habitat types provide a different set of resources that may or may not be of benefit to a species (Beck et al. 2001). A habitat can be defined as the place where an organism lives and interacts with the abiotic and biotic components of the environment (Begon et al. 1996). More comprehensively it can be defined as, “a spatially contiguous vegetation type that appears more or less homogenous throughout and is physiognomically distinctive from other such types” (Hutto 1985). The term ‘habitat complexity’ has been used synonymously with terms such as ‘heterogeneity’ and ‘diversity’, and signifies that different structural elements within a habitat form its complexity (Tokeshi and Arakaki 2012). In freshwater and marine systems, these elements may consist of living components, such as plant stems, leaves, aerial roots, moss patches, oyster reefs and coral reefs, and non-living parts, such as rocks, stones and submerged logs (Tokeshi and Arakaki, 2012).

Research on the functioning of near shore ecosystems has been conducted for over a century, and the nursery role of habitats became a gradually accepted concept since the first study on nursery areas by Hay (1905). Consensus on the meaning and implications of the nursery role concept had been lacking until Beck et al. (2001) put forward a nursery role hypothesis that provided a clear definition and explanation of the nursery role concept. The nursery role hypothesis proposed by Beck et al. (2001) states that, “a habitat is a nursery for juveniles of a particular species if its contribution per unit area to the production of individuals that recruit to adult populations is greater, on average, than production from other habitats in which juveniles occur.” Greater contributions to adult recruitment may occur from a combination of four factors, namely 1) higher abundance/density, (2) higher growth, (3) survival of juveniles and (4) movement to adult populations (Beck et al. 2001). The nursery-role hypothesis provides a framework for the assessment and valuation of various habitats as nurseries (Kraus and Secor 2005). Beck et al. (2001) argue that a definitive test of the nursery-role hypothesis requires a comparison between multiple habitat types, which provides a quantitative and direct method for assessing and comparing the nursery value of different habitat types.

Surprisingly, there have been few studies that have quantitatively assessed the nursery value of estuarine habitats for fish and invertebrate species (Bloomfield and Gillanders 2005). Initial studies focusing on the nursery role concept assessed estuaries as a whole (Beck et al. 2001, Heck et al. 2003, Dahlgren et al. 2006) Most studies evaluating nursery value have made comparisons between vegetated and un-vegetated habitats, but they have not evaluated the relative importance of different multiple vegetated habitat types (i.e., Briggs and O'Connor 1971, Koike and Nishiwaki 1977, Weinstein and Brooks 1983, Orth et al. 1984, Pollard 1984, Olney and Boehlert 1988, Connolly 1994). Some studies compared the fish communities occurring in different habitats in southern African estuaries. Paterson and Whitfield (2000) assessed the distribution of piscivorous fish in intertidal salt marsh creeks, neighbouring shallow water habitats and deep water habitats, and Paterson (1998) assessed the ichthyofaunal asemblages associated with intertidal salt marsh creeks and adjacent seagrass habitats in the Kariega Estuary. Hanekom and Baird (1984) compared the ichthyofaunal assemblage found in seagrass and non-seagrass habitats in the Kromme Estuary whilst Becker et al. (2010) used remote underwater video footage to compare the relative abundance of fish in reef, bare sand and reed beds in the East Kleinemonde Estuary, and Becker et al. (2012) compared the relative abundance of fish occurring in seagrass and sand habitats in the Bushmans Estuary.

Globally submerged macrophyte habitats (such as seagrass) are crucial for the development of nekton in many estuarine systems (Minello 1999, Minello et al. 2003). Submerged aquatic macrophytes function as direct and indirect sources of nutrition for fish using nursery areas (Sheppard et al. 2012). Seagrass habitats have been identified as critical nursery habitats for many juvenile fish species (Heck et al. 2003). In a meta-analysis of over 200 papers that addressed the nursery role of different habitat types in estuaries and coastal bays, Heck et al. (2003) found that the vast majority of studies confirmed that the abundance, growth and survival of fish species were significantly higher in seagrass habitats than in unstructured habitats such as mud and sand flats. Minello et al. (2003) undertook a meta-analysis study of varying densities of decapod crustaceans and juvenile fishes in different habitat types. Of the 32 papers assessed, seagrasses were ranked as the habitat with the highest fish densities; vegetated marsh edges, non-vegetated marsh, open water, and macroalgal habitats were found to have densities similar to each other, but higher than those of oyster reefs (Minello et al. 2003).

Vegetated communities in South African estuaries consist of seagrasses in the subtidal and lower intertidal zones, and salt marsh communities in the intertidal and supratidal zones (Jafta 2010). *Zostera capensis* is known to dominate the lower and middle reaches of permanently open estuaries where salinity is close to seawater (Talbot and Bate 1987). *Zostera capensis* may also occur in the upper reaches of permanently open estuaries where freshwater flow is low, whilst *Potamogeton pectinatus* and *Ruppia cirrhosa* are common in temporarily open estuaries where salinity is lower (Adams et al. 1999). Salt marsh communities in warm temperate estuaries in South Africa are generally dominated by *Spartina maritima* in the lower marsh zone (Jafta 2010). The middle marsh zone is dominated by *Sarcocornia perrenis, Triglochin bulbosa*, *Triglochin striata*, *Salicornia meyeriana*, *Cotula coronopifolia*, *Limonium* sp., *Bassia diffusa* and *Sueada inflata*, whilst *Sarcocornia pillansii*, *Puccinella angusta*, *Disphyma crassifolium* and *Plantago crassifolia* occur in the upper marsh zone (Jafta 2010).

When testing the nursery-role hypothesis, one should focus on species with separate juvenile and adult habitats, and all habitats that the juveniles use need to be assessed (Beck et al. 2001). In warm-temperate estuaries the sparid, *Rhabdosargus holubi* was the most abundant marine species recorded in both permanently open and temporarily open/closed estuaries (Vorwerk et al. 2001, Harrison 2005).

Rhabdosargus holubi, which is endemic to southern African waters, is distributed from the Berg Estuary on the southwest coast to Inhaca Island in the northeast (Whitfield 1998) and is considered to be entirely dependent on estuaries for the first year of life (Whitfield 1994). *Rhabdosargus holubi* cannot tolerate temperatures below 5°C and above 31°C and in South Africa is restricted mainly to the area between Cape Point and northern KwaZulu-Natal. *Rhabdosargus holubi* is replaced by *Rhabdosargus sarba* in the northeast (subtropical region) and by *Rhabdosargus globiceps* in the southwest (cool-temperate region) (Blaber 1973).

Rhabdosargus holubi are well adapted to estuaries, they are euryhaline and are tolerant of a wide range of salinity concentrations (0.7–70) and temperatures (10°–30°C) (Blaber 1973). *Rhabdosargus holubi* has an extended breeding season, with spawning occurring in the nearshore marine environment between July and February in the Eastern and Western Cape (Blaber 1973, Whitfield and Kok 1992, Whitfield 1998). Although the main recruitment period into south-eastern Cape estuaries is from August to April (Blaber 1974, Beckley 1983, Whitfield and Kok 1992), recruitment has been recorded throughout the year in the Knysna, Swartvlei and East Kleinemonde estuaries (Beckley 1983, Whitfield and Kok 1992, James et al. 2007). The continual recruitment of early juveniles into estuaries along the south-eastern Cape coast will have a buffering effect against recruitment failure (James et al. 2007). Postflexion larvae and early juveniles enter estuaries at a size range of between 6 and 15 mm standard length (Cowley et al. 2001, James et al. 2008). Most individuals move out to sea as juveniles once they have reached approximately 14 cm, after approximately one year (Whitfield 1988), but they are capable of remaining in closed estuaries until conditions are suitable for emigration (James et al. 2007). In a long-term study of the fish communities in the East Kleinemonde Estuary, some individuals remained trapped in the estuary for up to 23 months (James et al. 2007). Adults are found in the marine environment to a depth of approximately 50 m (Wallace et al. 1984).

Certain fish species are more closely associated with submerged vegetation than others (Sheppard et al. 2011). Juveniles of *R. holubi* often occur in the vicinity of aquatic plants as these habitats are thought to provide refuge from predators and support an abundant supply of invertebrates and epiphytes (Whitfield 1984, Cowley and Whitfield 2001). In the temporarily open/closed East Kleinemonde Estuary, decreases in the abundance and percentage contribution of *R. holubi* to the catch were recorded following the loss of submerged macrophytes from the system.

In a study of the fish community of the Kromme Estuary, *R. holubi* was found to be significantly more abundant ($P<0.05$) in seagrass areas than in bare sediment areas (Hanekom and Baird 1984). Similarly, Beckley (1983) found that *R. holubi* was the second most abundant species found in the seagrass beds of the Swartkops Estuary. *Rhabdosargus holubi* are known to ingest submerged aquatic macrophytes and digest the layer of epiphytic diatoms occurring on macrophyte leaves, as well as to prey upon a broad range of invertebrates (Blaber 1973). Underwater observations of *R. holubi* behaviour have revealed that during daylight hours they gather in shoals of between 10 and 400 individuals (Blaber 1973). They have been found to frequent and forage on the fringes of *R. cirrhosa* beds. At night they have been observed to sleep on the bottom surface amongst *R. cirrhosa* beds and are more dispersed, where they remain inactive from approximately 21h00 until dawn (Blaber 1973).

Rhabdosargus holubi has also been recorded in salt marsh habitats, but not in high numbers. In a study of the fish assemblage of salt marsh creeks in the Kariega Estuary, only 2% of the individuals caught were *R. holubi*. This species did, however, have the highest frequency of occurrence (Paterson 1998). Although *R. holubi* is regarded as a vegetation-associated species (Sheppard et al. 2011), it has also been recorded in other habitat types such as reed, reef and sand habitats (Becker et al. 2010).

Research aims and objectives

This study aims to quantify the nursery value of the dominant habitat types in the Bushmans Estuary for juvenile *R. holubi*. The research objectives include:

1) Determine the degree of habitat complexity and hence the suitability of two dominant vegetated estuarine habitats in the Bushmans Estuary as nursery areas for *R. holubi* using a number of complexity indices at a range of scales.
2) Determine the abundance and habitat use (behaviour) of the dominant habitats in the Bushmans Estuary for *R. holubi*.
3) Use stomach content analysis and dietary diatom composition to identify the important estuarine feeding habitats of juvenile *R. holubi.*

By achieving these objectives it is hoped that this study will significantly contribute towards a better understanding of the nursery function of estuarine habitats for *R. holubi* in the Eastern Cape.

CHAPTER TWO

STUDY AREA

Introduction

The Bushmans Estuary is located at the town of Boesmansriviermond in the warm-temperate Eastern Cape Province of South Africa (33°41'41"S; 26°39'48"E) (see Fig. 2.1) and is classified as a large, permanently open estuary (Day 1981).

Climatic conditions

The estuary is situated within a bi-modal rainfall region with peak rainfall periods occurring in March and October (Jafta 2010). Annual rainfall recordings in the Bushmans River catchment range between 300–400 mm in the upper reaches and 800–900 mm in the lower reaches, averaging approximately 717 mm annually (Reddering and Esterhuysen 1981, Bornman and Klages 2004). Average monthly temperatures range between 21.7 °C in January and 15.4 °C in July with maximum temperatures of 26.3 °C in January and February and minimums of 10.2 °C in July (Bornman and Klages 2004, Jafta 2010).

Abiotic characteristics

The estuary is approximately 40 km in length and 120–300 m wide and has a catchment area of 2675 km^2 (Day 1981). It is a meso-tidal system, with a tidal range of less than 2 m (Becker et al. 2012). Mean annual run-off has been recorded at approximately 38 $\times 10^6$ m^3 (DEAT 2001). Surface and deep water temperatures have been found to be similar, ranging between 18.7°C and 22.7°C (James and Harrison 2010). Water temperatures decrease during high and spring high tides when cold, marine water enters the estuary, whilst temperatures are higher where freshwater enters at the head of the estuary (Jafta 2010). Water depths range between 1.1m in the middle reaches and 4 m at the tidal head (James and Harrison 2010). The estuary is a freshwater deprived system with salinity from the lower to the upper reaches of the estuary analogous to that of seawater (30–35 PSU) (Day 1981, Whitfield et al. 1994, James and Harrison 2010) and largely characterized by the absence of a salinity gradient, except for periods of small flooding (Jafta 2010). During droughts, the salinity may exceed 35 ppt (Jafta 2010). The combination of low current speeds, high rates of sedimentation and strong tides has resulted in low turbidity (1.5–6 NTU) and clear waters in the lower reaches of the estuary, especially at high and spring high tides (Becker et al. 2010). James and Harrison (2010) found dissolved oxygen to range between 5.8 and 7.4 mg.l^{-1}, although Jafta (2010) has indicated that dissolved oxygen levels periodically neared hypoxic levels of 2 mg.l^{-1} (Jafta 2010). pH has been found to range between 7.7 and 8.0 (James and Harrison 2010).

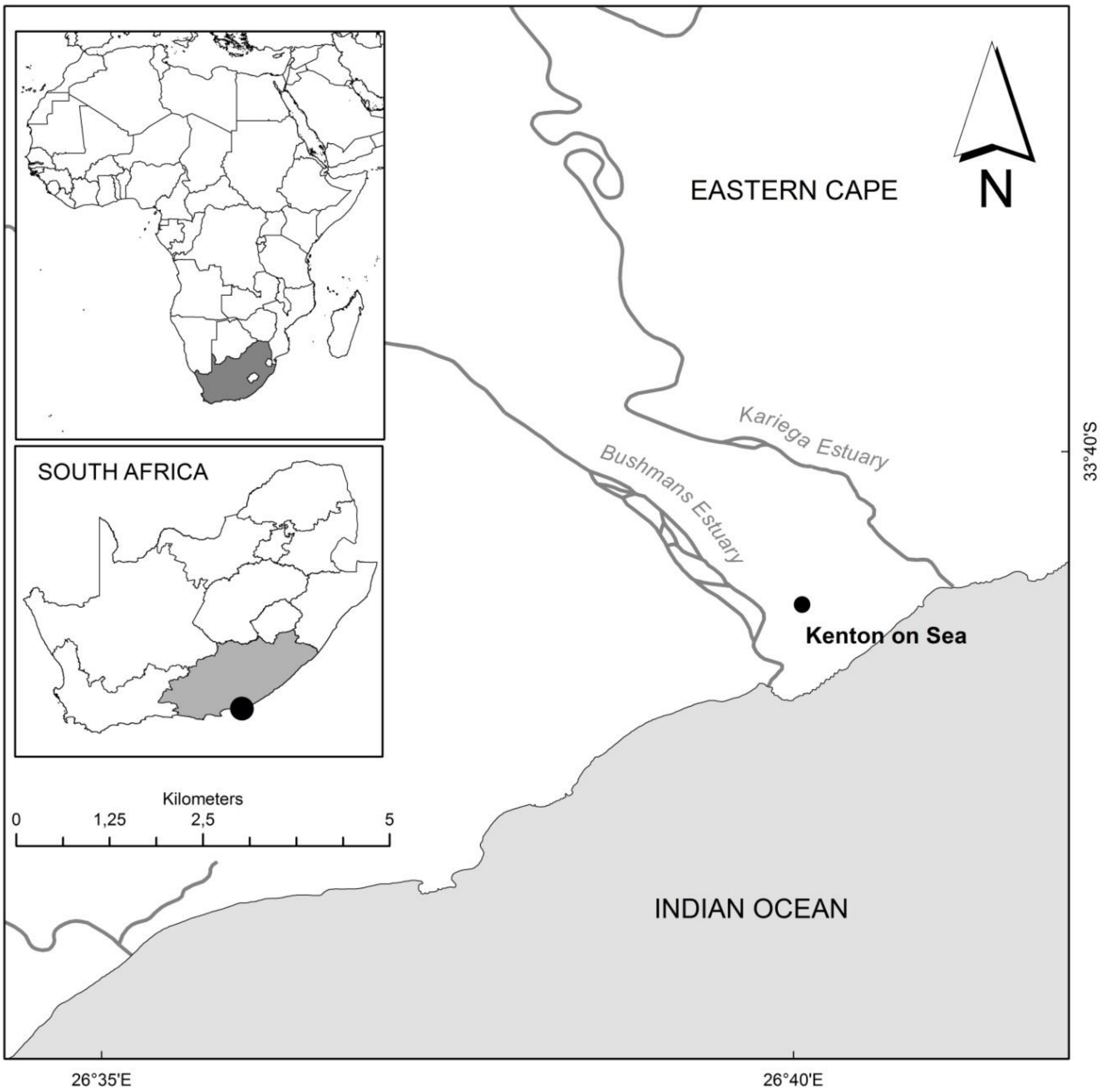

Figure 2.1: The continental, regional and local geographical location of the Bushmans Estuary

Major botanical communities

The Bushmans Estuary is dominated by several vegetated and unvegetated habitats, namely sand and mudflats, submerged macrophyte beds, emergent salt marsh beds and reeds and sedge communities (see Fig. 2.2). Jafta (2010) calculated the areal cover of the habitat types within the Bushmans Estuary in 2004. Submerged macrophytes, salt marsh, reed and sedges, and sand

and mud banks covered an area of 25.6 ha, 72.9ha, 0.8 ha and 58.8 ha respectively. Along the full length of the estuary, submerged macrophytes, salt marsh, reed and sedges, and sand and mud banks covered an area of 44.6 ha, 126 ha, 16.8 ha and 59.2 ha respectively in the lower reaches (Jafta 2010).

Zostera capensis is the dominant submerged macrophyte in the Bushmans Estuary and is prevalent at the subtidal zone in the lower, middle and upper reaches of the estuary (Jafta 2010). There has been a reported expansion of *Z. capensis* in the Bushmans Estuary to upstream sites. This has been attributed to increased sedimentation and higher salinity concentrations in the middle and upper reaches of the estuary (Jafta 2010). Low flows in the estuary of between $0.1m.s^{-1}$and $1m.s^{1}$ are considered to be conducive to the establishment and growth of *Z. capensis*. *Ruppia cirrhosa* has been recorded in the calm upper reaches of the estuary (Jafta 2010).

The emergent salt marsh community has been observed in the intertidal and supratidal zones. It is dominated by *S. maritima* in the lower reaches and by *Sarcorcornia* spp. in the upper reaches, whilst *Sarcocornia* occurs as a co-dominant with *S. maritima* in the middle reaches (Jafta 2010). *S. maritima* has been found to constitute approximately 47% of the total salt marsh area (Jafta 2010). *Triglochin* sp, *Limonium linifolium* and *Disphyma crassifolium* make up the rest of the salt marsh community, specifically in the lower reaches (Jafta 2010). Jafta (2010) reported a decrease of 20% of the areal cover of *S. maritima* between 1995 and 2008, possibly due to changes in elevation as a result of sedimentation, causing a decrease of intertidal area for *S. maritima* and an increase in supratidal area for other salt marsh species (Jafta 2010).

Isolated mixed freshwater/brackish wetland communities consisting of reeds and sedges occur sporadically along the length of the estuary. The reeds and sedge communities colonize the banks of the estuary in intertidal and subtidal zones where there is freshwater input (Jafta 2010, Prinsloo 2012). These small communities are characterized by *Phragmites australis* and *Typha capensis* reeds and the sedge *Bolboschoenus maritimus* in the lower and middle reaches (Jafta 2010). Individual and mixed stands of *P. australis* and *B. maritimus* are also present in the middle reaches (Jafta 2010). In the upper reaches, the reed and sedge communities consist of single stands of *P. australis* and mixed stands of *P. australis* and *B. maritimus* (Jafta 2010).

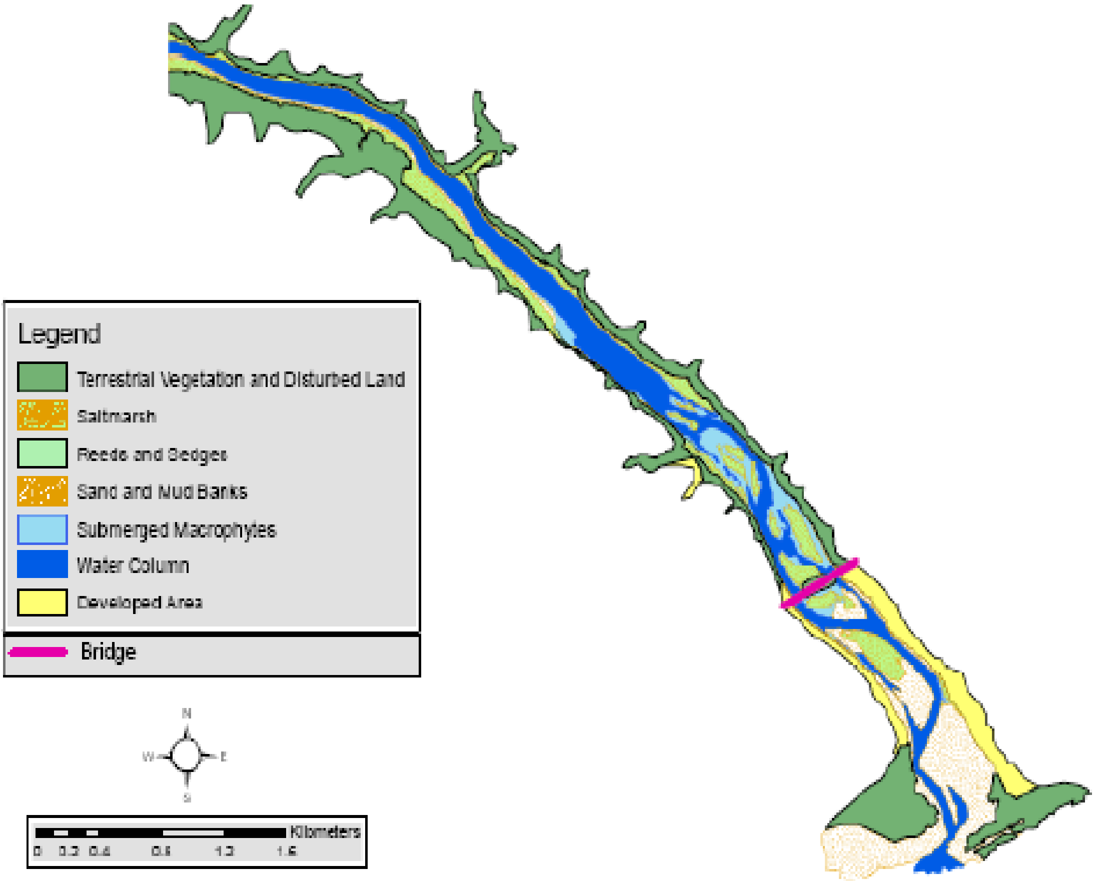

Figure 2.2: Distribution and cover of various habitat types in the lower reaches of the Bushmans Estuary in 2004 (taken directly from Jafta 2010)

Anthropogenic impacts

The Present Ecological Status of the Bushmans Estuary is currently ranked in Category B, indicating an estuary that is largely in its natural state but has undergone small changes to its natural habitats and biota (van Niekerk et al. 2015). However, there are multiple anthropogenic impacts of concern in the estuary. The estuary has been negatively impacted upon by reductions in freshwater supply due to intensive agricultural activity and the construction of 17 registered impoundments, including the New Years River Dam that has a capacity of 4.7×10^6 m^3 (Whitfield et al. 1994, Jafta 2010). Furthermore, other unregistered impoundments with unknown storage capacities are also present in the catchment (Jafta 2010). The mean annual run-off in the catchment is considered low, and hence reductions in the freshwater supply are of concern to the health of the estuary. Furthermore, there is a predicted 50% decline in the freshwater available in the catchment by 2025 (Papadopoulos 2006) and hence, the ecological impacts associated with decreased freshwater supply may intensify.

Jafta (2010) indicated that there is poor water clarity in the estuary, specifically in the upper and middle reaches, due to high sediment load consisting of suspended silt and solids. Jafta (2010) suggested that this has been caused by sediment entering the estuary from runoff and wind from poorly vegetated degraded land and agricultural land neighbouring the Bushmans Estuary. Increased silt in the water column, combined with low river flow, has resulted in increased silt deposition, possibly extending the intertidal area (Jafta 2010).

Sewerage spills into the estuary has also been cited as a concerning anthropogenic factor (Jafta 2010). There have been multiple reports of septic tanks overflowing during peak holiday season in the lower reaches. Nutrient input from developments neighbouring the river and the waste water treatment works in the catchment influence the nutrient budget of the estuary (Jafta 2010). Pesticides, herbicides and fertilizers entering the estuary during rainfall events may also be contributing pollutants to the estuary (Jafta 2010).

Recreational activity is common in the estuary, especially in the lower reaches. Increases in the recreational activity have caused and may continue to cause disturbances. Walking and digging for bait in *Z. capensis* beds is suspected to have a detrimental impact on submerged aquatic macrophytes such as *Z. capensis* (Jafta 2010). Boating has been found to be a popular activity in the estuary and the propellers of boats re-suspend sediment and can cause physical damage to macrophytes (Jafta 2010).

CHAPTER THREE

THE SUITABILITY OF ESTUARINE VEGETATED HABITATS AS NURSERY AREAS IN THE BUSHMANS ESTUARY FOR JUVENILE *RHABDOSARGUS HOLUBI*

INTRODUCTION

Despite the well accepted view that habitat complexity plays an important role in faunal abundance and richness, biotic structure and consequently ecosystem functioning (Kovalenko et al. 2012), there is no standardized methodology for comparing habitat complexity between habitats or ecosystems (Kovalenko et al. 2012). In the aquatic environment, studies attempting to relate species abundance and diversity to habitat complexity initially simply recorded the presence or absence of vegetation (Heck and Wetstone 1977). Following from this approach, studies began to focus on plant structural elements such as biomass, stem density, stem length (also canopy height) and leaf length to describe habitat complexity within and between habitats. Seagrass biomass, as an indicator of habitat complexity, has been related to an increase in the biomass of invertebrate species (Heck and Wetstone, 1977) and biomass, abundance and richness of fish species (Wyda et al. 2002). For small invertebrates such as amphipods, this approach proved less effective in seagrass and green algae because high biomass did not always result in high abundance; this was attributed to predation through the spaces between fine branches (Stoner 1982).

Measuring stem density has also been a popular proxy for measuring habitat complexity. Increasing stem density of salt marsh (measured as stems per unit area) has been found to decrease predation rates of the snail *Melampus bidentutus* and the amphipod *Orchestia griflus* by the Killifish *Fundus heteroclitus* (Vince et al. 1976). For seagrass, stem density has been correlated with the survival of crabs *Callinectes sapidus* (Hovel and Lipcius 2001). Gratwicke and Speight (2005) found that canopy height of fleshy algae, seagrass, mangrove, sand, rubble and reef habitats was an accurate predictor for fish abundance. Using several plant elements, Gullstrom et al. (2008) found that in conjunction with stem density and biomass, canopy height played a significant role in structuring patterns and variability of seagrass associated fish assemblages.

Although many studies have successfully used selected structural plant elements to measure habitat complexity, such methods oversimplify the true complexity of vegetated systems, as complexity is not only defined by the amount of available habitat or the number of structures in a habitat but also by the shape of plant structures (McAbendroth et al 2005). Moreover, individual or a combination of structural plant components cannot be used to directly compare complexity between habitat types as these are not drawn from obvious, direct comparisons between different plant species (Warfe et al. 2008, Kovalenko et al. 2012). Stem and leaf shape and other plant attributes can differ significantly in shape and size between plant species and thus, direct comparisons of complexity between habitat types using plant structural components can be misleading (Kovalenko et al. 2012).

Dimensionless index approaches have been devised as an improved method for quantifying habitat complexity between different vegetated habitats. Bartholomew et al. (2000) proposed that total cover provided by a habitat is a good appraisal for habitat complexity because it quantifies the total cross-sectional area provided by a habitat type for an organism to conceal itself from predators. Dividing the total plant cover (C_t) by the area of the habitat (A_t) gives the total amount of cover provided within a given area (C_t/A_t), allowing for comparisons between habitats (Bartholomew et al. 2000). C_t/A_t has been used as a dimensionless index in laboratory experiments; for example, dowels were used as analogues of the *S. alterniflora* salt marsh stems in an assessment of prey survival of a number of amphipod species predated by the large fish *Fundulus heteroclitus* (Bartholomew et al. 2000). Later similar trials used flat green ribbons to provide structural cover (Bartholomew 2002a). No known studies have attempted to quantify C_t/A_t of macrophytes in the field.

Dibble et al. (1997) devised a habitat complexity index for macrophytes known as the index of interstitial space (ISI) for comparing complexity by measuring the length and frequency of interstitial spaces (open space between stems and leaves) along vertical and horizontal axes, providing a measurement of the degree to which space is broken up by macrophyte structures. This method was successfully used by Dibble and Thomaz (2006) to measure the spatial complexity between eight aquatic plant species in the Upper Paraná River floodplain in Brazil. This complexity index has provided an approach for comparisons between habitats of different vegetation types and has enabled scientists to test the Heck and Wetstone (1977) hypothesis that increased habitat complexity is related to the increased provision of living space and niche space for organisms (Kovalenko et al. 2012).

The use of fractal dimensions has become increasingly popular in recent complexity studies (Thomaz et al. 2008, Tokeshi and Arakaki 2012). A fractal describes an object's configuration by its inert geometry through analysing the object's surface and shape (Kostylev et al. 2005, Warfe et al. 2008) and describes its complexity by replicating the extent to which apparent lengths change as measurement scales change (Sugihara and May 1990). An increase in the fractal dimension (D) implies a higher degree of intricacy or convolution of an object and hence a higher degree of complexity (Kostylev et al. 2005, Warfe et al. 2008). Although it is accepted that simple geometry cannot be used to explain the shape of naturally occurring objects because their shapes are often irregular, convoluted and highly complex and vary at different spatial scales (Sugihara and May, 1990), a shared trait between natural objects and fractals is that both have relevant features at a range of scales (Sugihara and May 1990).

Naturally occurring objects of high fractal dimensions offer a greater variety of spaces at different scales. When describing an object on a two-dimensional surface at a single scale, the amount of area available for species of any size is uniform. However, as a fractal dimension increases, there is a decrease in the amount of area available (Kostylev et al. 2005). Therefore, there are a greater number of microhabitats available for smaller organisms and fewer microhabitats available for larger organisms. Fractals are related to habitat heterogeneity and niche space as fractals describe unequal share of available space for animals of different sizes. It is critical that the scale at which an object is analysed is relevant to the scale of the organism in question (Kostylev et al. 2005). Sugihara and May (1990) were the first to apply fractals to ecology to answer questions relating complexity to naturally occurring objects in ecological systems. Since then fractal geometry has been used to measure the effect of plant complexity on invertebrate richness and density in marine habitats (e.g., Gee and Warwick 1994, Davenport et al. 1999, McAbendroth et al. 2005, Thomaz et al. 2008). In terms of fishes, one study by Thistle et al. (2010) used fractals to compare the habitat complexity of *Z. marina* seagrass beds in relation to the density of three species of juvenile cod (*Gadus morhua, Gadus ogac* and *Urophycis tenuis*). They found that eelgrass sites of intermediate spatial complexity provide juvenile fish with both optimal protective cover and opportunity to feed.

Kovalenko et al. (2012) suggested that quantitative methods for assessing habitat complexity should be more broadly used in future studies to enhance the comprehension of the mechanistic role of habitat structure whilst dimensionless indices and fractals should be used for direct between-habitat comparisons of habitat complexity. Furthermore, Kovalenko et al. (2012) suggested that in order to make comparisons of complexity between habitats, multiple approaches should be incorporated into complexity studies to provide a more rigorous assessment of habitat complexity. For example, plant element density and plant shape can evoke different responses from predators, highlighting the importance of analysing both components in complexity studies (Warfe and Barmuta 2004).

Aims and objectives

The main objective of this chapter is to determine the degree of habitat complexity of two estuarine habitats in the Bushmans Estuary. If it is assumed that the more complex habitats are more effective for predator avoidance, these results may provide insight as to which habitat will provide a better nursery habitat for *R. holubi*.

MATERIALS AND METHODS

Plant structural elements

Separate beds of *Zostera capensis* seagrass and *Spartina maritima* salt marsh were selected in the lower reaches of the Bushmans Estuary for sampling. Sampling was undertaken in spring, summer, autumn and winter in September 2013, February, May and July 2014 during spring low tide cycles to measure seasonal changes in plant structure, above- and below-ground biomass, stem density and stem length measurements. In each habitat type, three transects were set up across the width of the habitat, perpendicular to the shore edge (see Fig. 3.1), with each transect measuring between 100 m and 220 m in length depending on the width of the vegetated habitat.

Sampling for above- and below-ground biomass and stem density was conducted every 20 m along each transect. Below-ground biomass was removed to a depth of 60cm. Biomass was removed from the sediment within a 0.0625 m^2 placed quadrat at 20 metre intervals. Samples were placed in a 3 mm sieve and rinsed with estuarine water to remove the attached sediment. In the laboratory, above- and below-ground biomass were separated, and the number of stems counted and recorded. The samples were then oven dried at 70°C for 8 hours and weighed. Stem length was measured in the summer only. The stem length of 10 *S. maritima* macrophytes (base of stem to highest leaf tip) and *Z. capensis* shoots (from rhizome to leaf tip) per quadrat in each transect was measured to the nearest mm.

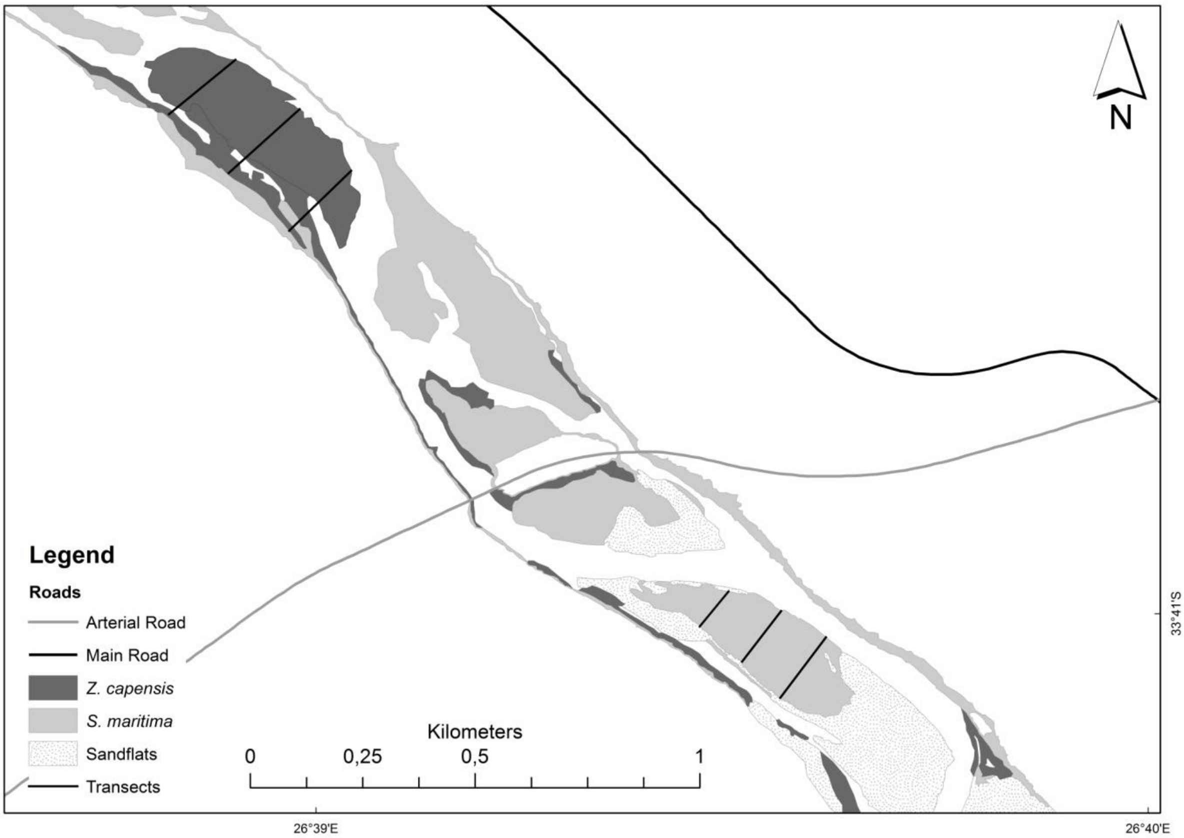

Figure 3.1: The location of the *Z. capensis* seagrass and *S. maritima* salt marsh beds and transects in the selected habitats for sampling in the lower reaches of the Bushmans Estuary

Dimensionless complexity indices

Dimensionless complexity indices were measured once off in autumn as opposed to seasonally.

A total cover per habitat area index (C_t/A_t) (Bartholomew 2002a) was calculated by estimating the average total cover provided in each habitat type per square metre. The total area of each habitat was not measured because a per square metre measurement was preferred for a direct between habitat comparison (Bartholomew et al. 2000). The total area of 25 individual *Z. capensis* and *S. maritima* plants of varying stem length was measured using a plant area meter combined with WINDIAS version 3.2 software. A regression between stem length and plant area was calculated to express the relationship between the two variables. Stem length of 10 individuals and stem density counts (obtained in summer in each quadrat per transect in each habitat) was used to estimate the total cover area per square meter in each plot in each transect using the regression equation between known stem length and plant area. Measuring the total cover area of an individual plant ensures that the greatest area that structure can produce is quantified (Bartholomew 2002a). The total cover provided by each habitat per square meter was averaged for each transect, providing a total cover estimate per habitat area index (C_t/A_t).

The interstitial spatial index (ISI) was used to measure the nature of spaces between plant structures of *Z. capensis* and *S. maritima*. ISI was quantified by taking underwater photographs of individual and multiple *Z. capensis* and *S. maritima* stems in the field in its natural state using a GoPro Hero 3 underwater camera. A marked whiteboard was placed 15 cm behind the macrophytes to provide a depth of view, improve contrast and provide scale. Images were taken at full spring high tide at a low turbidity (NTU = 2.3) for high visibility. The white board was positioned parallel to the tide to avoid changes due to current speed and retain natural macrophyte structure. In order to avoid the possibility of fine-resolution measurements of interstitial space that might overestimate habitat complexity through measuring plant components that could be unusable to macro-organisms (Kovalenko et al. 2012), ISI was calculated at three magnifications to account for different scales of complexity, as suggested by Dibble and Thomaz (2006). Another benefit of choosing three distances is to test if the ISI index is variable or similar at different distances. Hence, photographs were taken at three distances from the vegetation edge. This resulted in three magnifications – low, intermediate and high representing an area of 100 cm^2, 50 cm^2 and 25 cm^2 respectively. The low magnification photograph captured a large number of whole macrophytes, the intermediate magnification photograph captured fewer whole macrophytes and the high magnification photograph focused on an individual macrophyte. Three replicate photographs were taken at each magnification. The default fish eye distortion effect of the images taken with the GoPro camera was corrected using Adobe Acrobat After Effects CS6 version 11.0.2. Images were analysed in ImageJ version 1.48, where they were converted to binary images. The brightness and contrast of the images were delicately modified in ImageJ to improve the profile of plant structures.

Each image was equally divided into three horizontal and three vertical axes by superimposing line transects and a measuring grid onto the image (Dibble and Thomaz 2006). The line intercept method was used to measure the length and frequency of each axis in each photo to the nearest millimetre as carried out by Dibble and Thomaz (2006) (Fig. 3.2). These measurements provided: the mean frequency of interstices per metre intercepted along a horizontal axis (f_h); the mean length of all interstices present along a horizontal axis (l_h); the mean frequency of interstices intercepted along a vertical axis (f_v); and the mean length of all interstices along a vertical axis (l_v) (Dibble et al. 1997). For each magnification, the frequency of interstices were multiplied by the appropriate length to get a mean number of interstices per metre (Dibble and Thomaz 2006). The above calculated values were then used to calculate the index of spatial complexity (ISI) where: $I_{hv} = (f_h/l_h) + (f_v/l_v)$. The greater the I_{hv} value, the higher the frequency and smaller the length of interstices between plant structures and hence, the higher the spatial complexity.

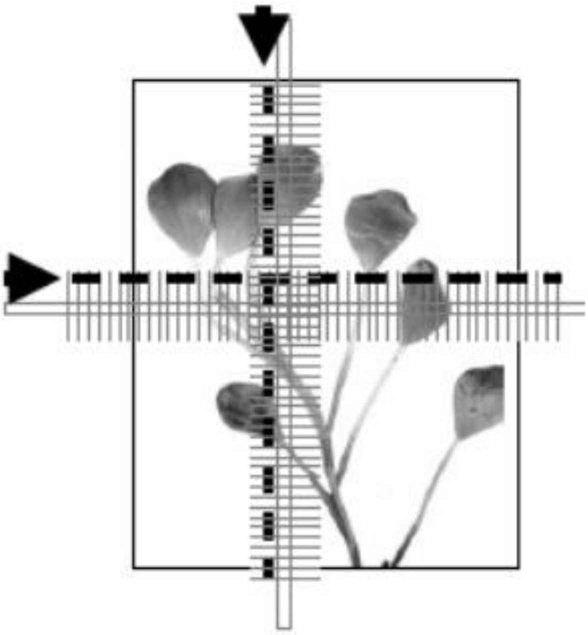

Figure 3.2: Depiction of the orientation of vertical and horizontal axes across a macrophyte analogue used to calculate the length and frequency of plant interstices. Image: Dibble and Thomaz (2006)

Fractal dimensions

The high and intermediate magnification (50 cm^2 and $25cm^2$) binary images used to calculate ISI were also used to quantify the fractal dimension (*D*) of *S. maritima* and *Z. capensis* stands. The intermediate magnification photos (30 cm^2) and high magnification photos (10 cm^2) were used to quantify the fractal dimension of multiple, whole macrophytes and the fractal dimension of a macrophyte focusing on the stem and leaves present in the field of view respectively. The low magnification photo (60 cm^2) used for ISI analysis was abandoned as the magnification and clarity of the image was too low and the density of macrophytes too high in the photos of both vegetation types. Fractals of both sets of photos reached a maximum value of two.

ImageJ v1.47 software was used to perform the fractal analysis. ImageJ employs the box-counting grid method developed by Sugihara and May (1990) and was used to calculate the fractal dimension of the area (D_A) and perimeter (D_P) of shapes (McAbendroth et al. 2005). The fractal dimension is estimated by calculating the slope of log N(s) plotted against log (1/s), where s is the scale used in the analysis and N(s) is the amount of objects found at that scale (Thomaz et al. 2008). The number of squares used to divide the space of the whole image was 2, 4, 6, 8, 12, 16, 32, 64, 128 and 256 (Dibble and Thomaz 2009). The two magnifications used in the fractal analysis, intermediate and high magnification, provided two scales that may be of use to different sized *R. holubi*.

Data analysis

Shapiro–Wilkes and Levene's tests were used to ensure that the assumptions of normality and homogeneity of the variances were met. Data was square-root transformed or cube root transformed where necessary. A repeated measures two-way ANOVA was conducted to assess seasonal changes in above-ground biomass, below-ground biomass and stem density between *S. maritima* and *Z. capensis*. A *post hoc* Tukey HSD test was used to test for interactions in seasons between vegetation types.

RESULTS

Spartina maritima salt marsh and *Z. capensis* seagrass showed little variation in above-ground biomass between spring, autumn and winter but did show marked increases in summer, with *S. maritima* showing the greatest growth (Fig. 3.3). The above ground biomass of *Z. capensis* was 42.6 $g.m^{-2}$ and 67.6 $g.m^{-2}$ in spring and summer, respectively and 33.5 $g.m^{-2}$ in autumn and 33.6 $g.m^{-2}$ in winter. The above ground biomass of *S. maritima* was 79.0 $g.m^{-2}$ and 155.5 $g.m^{-2}$ in spring and summer, respectively and was 61.2 $g.m^{-2}$ in autumn and 62.4$g.m^{-2}$ in winter.

The below ground biomass of *Z. capensis* was 92.2 and 86.6 $g.m^{-2}$ in spring and summer, respectively and decreased to 63.2 $g.m^{-2}$ in autumn and 70.5 $g.m^{-2}$ in winter (Fig. 3.4). The belowground biomass of *S. maritima* was 116.3 $g.m^{-2}$ and 102.8 $g.m^{-2}$in spring and summer, respectively and decreased to 73.8 $g.m^{-2}$ in autumn and increased to 119 $g.m^{-2}$ in winter.

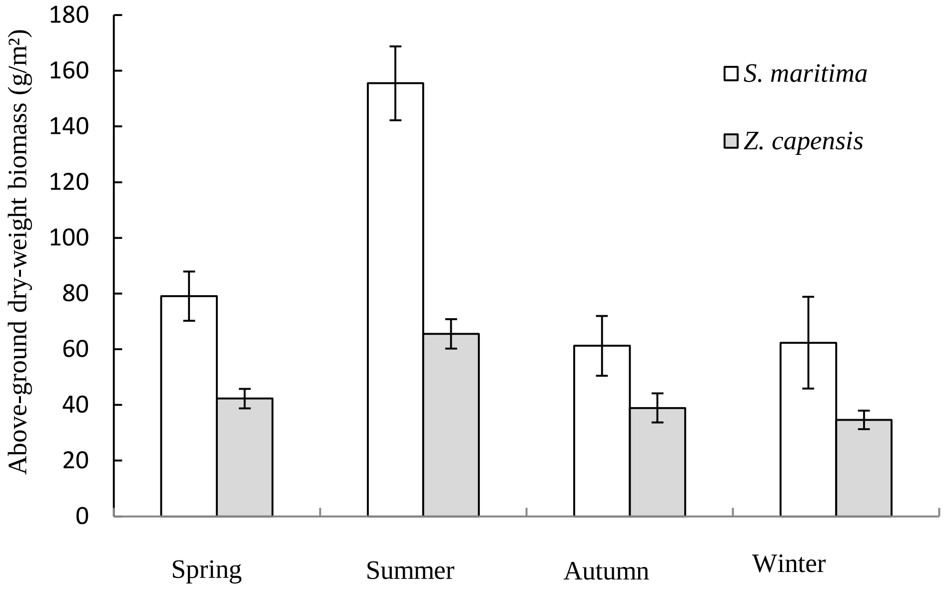

Figure 3.3: Comparison of above-ground biomass of *S. maritima* (n=24) and *Z. capensis* (n= 36) between and within seasons. Error bars represent standard error.

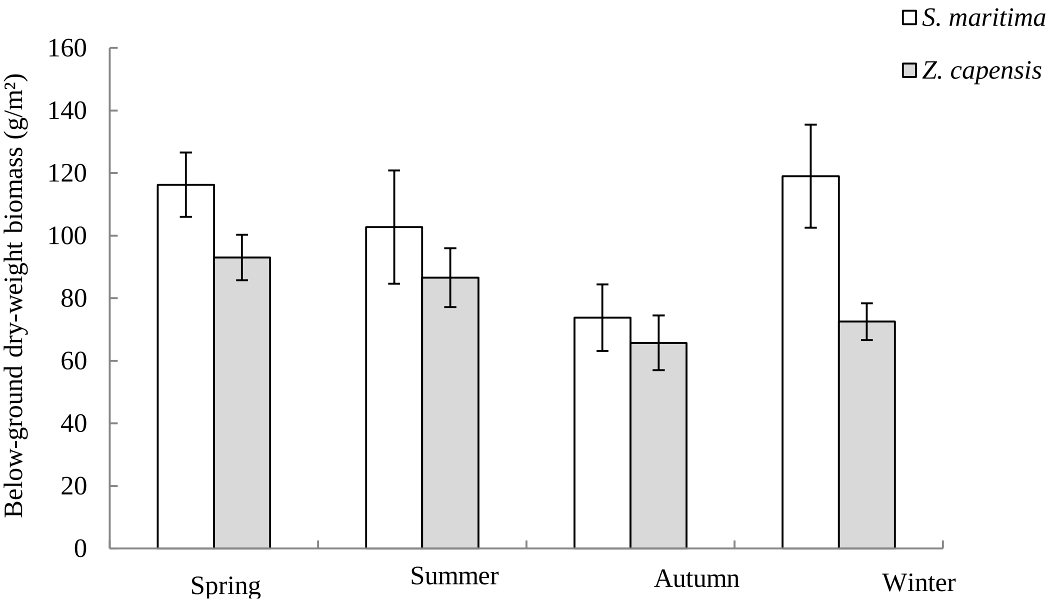

Figure 3.4: Seasonal changes in below-ground biomass of *S. maritima* (n=24) and *Z. capensis* (n= 36). Error bars represent standard error.

There was a general significant difference in above-ground biomass (F=88.3, P<0.01) and below-ground biomass (F=60.7, P<0.01) between *S. maritima* and *Z. capensis*. *Post hoc* analysis revealed that above-ground biomass of *S. maritima* was significantly higher than *Z. capensis* in spring (F=227.55, P<0.01), summer (F=227.55, P<0.01), autumn (F=227.55, P<0.05) and winter (F=227.55, P<0.01). *Spartina maritima* below-ground biomass was also significantly higher than *Z. capensis* for all seasons (spring - F=227.70, P<0.001), (summer - F=227.79, P<0.001), (autumn - F=227.79, P<0.001) and (winter - F=227.79, P<0.001).

The stem density of *Z. capensis* ranged from 873 m^2 in summer to 540 m^2 in winter whilst *S. maritima* stem density ranged from 193 m^2 in summer to 100 m^2 in autumn. Stem density was significantly higher in *Z. capensis* in spring (F=221.94, P<0.01), summer (F=221.94, P<0.01), autumn (F=221.94, P<0.01) and winter (F=221.94, P<0.01) compared to *S. maritima* (Fig. 3.5).

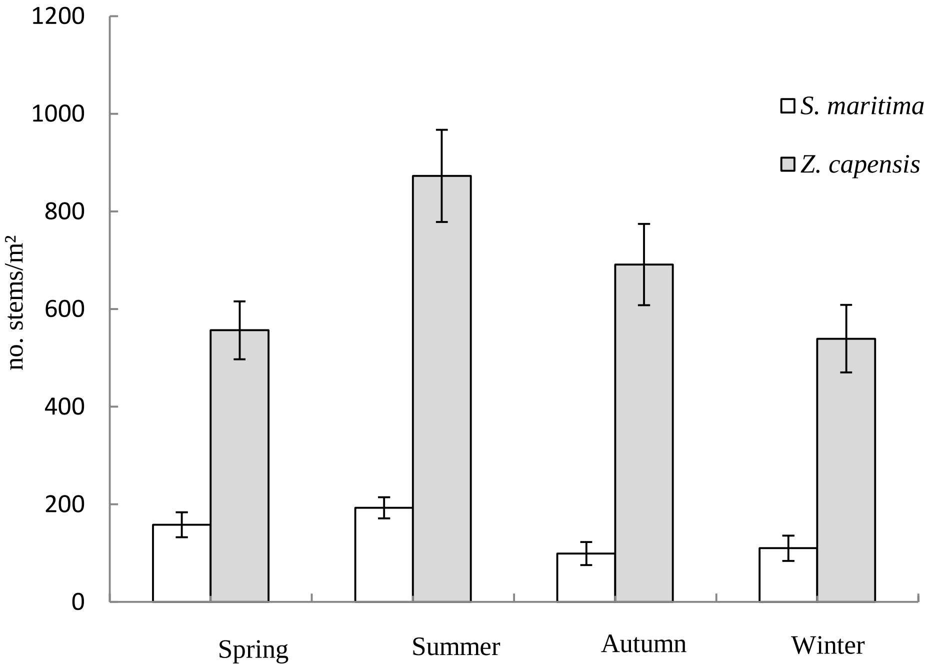

Figure 3.5: Seasonal changes in stem density of *S. maritima* (n=24) and *Z. capensis* (n= 36). Error bars represent standard error.

Spartina maritima stem length was found to be significantly higher in than *Z. capensis* stem length (t=4.6, P<0.001), with an average difference of 74 mm between the two vegetation types (Fig. 3.6).

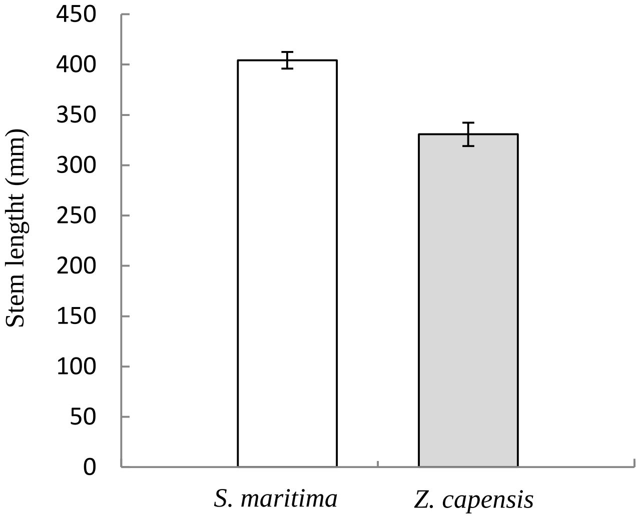

Figure 3.6: Average stem length of all plots in summer of *S. maritima* (n=24) and *Z. capensis* (n=36). Error bars represent standard error.

Results of plant complexity indices varied in degrees of complexity between *S. maritima* and *Z. capensis* (Table 3.1). Dimensionless complexity indices consistently indicated that habitat complexity was considerably higher in *Z. capensis* than in *S. maritima*. C_t/A_t averaged 1.04 m^2/m^2 in *Z. capensis* compared to 0.76 m^2/m^2 in *S. maritima.* ISI scores were higher in *Z. capensi*s than *S. maritima* for each of the three magnifications. ISI averaged 2.43 in *S. maritima* and 12.91 in *Z .capensis* at low magnification, 2.92 and 7.99 at intermediate magnification and 2.12 and 7.14 at high magnification. The fractal dimension values were higher in *Z. capensis* than in *S. maritima* at both magnifications (Table 3.1). *Spartina maritima* had a higher fractal dimension at high magnification (D=1.27) and lower at intermediate magnification (D=1.42). The fractal dimension of *Z. capensis* increased from 1.4 at intermediate magnification to 1.57 at high magnification.

Table 3.1: Comparison of the structural and dimensionless complexity indices of *S. maritima* and *Z. capensis* with standard errors (SE).

Complexity index	*S. maritima*	SE	*Z. capensis*	SE
Simple structural components:				
Aboveground biomass (g.m^{-2})*	87.7 (n=96)	7.57	44.9 (n=144)	2.42
Summer canopy height (cm)	40.4 (n=180)	8.30	33.07 (n=180)	11.5
Stem density (no.m^{-2})*	141.61 (n=96)	25.31	761.11 (n=144)	40.08
Dimensionless indices:				
Ct/At (m²/ m²)	0.76 (n=3)	0.01	1.04 (n=3)	0.12
Interstitial Spatial Index (ISI)				
Low magnification	2.43 (n=3)	0.07	12.91 (n=3)	0.63
Intermediate magnification	2.92 (n=3)	0.21	7.99 (n=3)	0.19
High magnification	2.12 (n=3)	0.1	7.14 (n=3)	0.05
Fractal dimension (D):				
Intermediate magnification (50cm^2)	1.27 (n=3)	0.01	1.4 (n=3)	0.02
High magnification (25cm^2)	1.42 (n=3)	0.07	1.57 (n=3)	0.01

* averaged for all seasons

DISCUSSION

Plant architectural elements and complexity indices were used as different approaches to assess habitat complexity of the dominant vegetation types in the Bushmans Estuary. The different aspects of plant architecture described in this study together provide an overall account of habitat complexity. The plant structural elements revealed varied results. Above ground biomass indicated that complexity is significantly higher in *S. maritima* than in *Z. capensis*, canopy height indicated a small difference in stem length, and stem density was more than 5 times greater in *Z. capensis* than in *S. maritima*. Each dimensionless index consistently indicated that complexity was higher in *Z. capensis* than in *S. maritima* at various magnifications. In *S. maritima*, ISI indicated that complexity was similar at each magnification whilst the fractal dimensions indicated that complexity was higher at high magnification than at low magnification. However, ISI indicated that complexity in *Z. capensis* was higher at low magnification than at high magnification whilst the fractal dimension indicated that complexity in *Z. capensis* was higher at high magnification than at low magnification.

Plant structural elements

There are a variety of factors that determine the stem density, biomass and overall productivity of salt marshes and seagrasses in coastal ecosystems. *Zostera capensis* above-ground biomass in the Bushmans Estuary is comparable to that of other studies in South Africa (Appendix A1) and exhibits a large degree of seasonality. In contrast, at the northern limit of its distribution in Inhaca Island, Mozambique, de Boer (2000) found above-ground biomass of *Z. capensis* to be 2-3 times lower than in this study. This result can be explained by the fact that the study was conducted in an intertidal bay at the northern most limit of the distribution of *Z. capensis*, where environmental variables are the most restricting to growth (de Boer 2000). In the northern hemisphere, above-ground biomass of *Z. marina* in the Yealm Estuary, U.K, has an even larger degree of variability than *Z. capensis* (Attrill et al. 2000).

The depth of the water, degree of water transparency and consequently the amount of light attenuation are thought to be the main factors that control the productivity of seagrasses such as *Z. capensis* (de Boer 2000, Ralph et al. 2007). The presence of seagrasses has been found to increase the deposition of suspended sediment, thereby decreasing turbidity and increasing light penetration and productivity (Carr et al. 2010). Other environmental factors such as temperature, physical disturbances, nutrient availability, cloudiness, water turbulence and herbivory also play an important role in controlling productivity (Agawin et al. 2001). However, these processes are complex, and various factors may cause different responses in different seagrass species (Agawin et al. 2001). *Zostera capensis* has been found to be limited by temperature, with temperatures less than 10°C known to limit the growth rate (Edgecumbe 1980).

The average above-ground biomass of *S. maritima* salt marsh recorded in this study of 87.7 $g.m^{-2}$ was more than six times lower than *S. maritima* above ground biomass in the Iberian Peninsula in Spain (Castillo et al. 2008). Above-ground biomass of *S. alterniflora* found in Parangua Bay is more comparable to that of *S. maritima* in this study (da Cunha Lana et al.

1991). This illustrates the large degree of global variability in *Spartina* sp. above-ground biomass. High accretion rates of fertile deposits and subsequent increases in nutrient supply and decreased flooding stress have been noted as the main factors explaining this (Castillo et al. 2008).

High variability within *S. maritima* beds is common, indicating the importance of the scale used in sampling, as larger sampling areas are more likely to account for variability in above- and below-ground biomass (Zedler 1983, Castillo et al. 2008). Nitrogen, soil aeration, soil drainage, sulphide concentration, soil salinity and temperature are factors that control productivity in salt marshes (de Leeuw et al. 1990). Sediment supply and deposition and the subsequent provision of nutrients such as nitrogen, iron, manganese and phosphorus in salt marsh systems have been found to be major determinants of productivity and plant regeneration in salt marshes in the Mississippi River (DeLaune et al. 1990). The rate of exchange between tidal and interstitial water (the degree of soil water drainage) proved to be a major determinant of stem density and above-ground biomass in *S. alterniflora* salt marshes in Georgia, USA (Wiegert et al. 1983). It is also known that changes in these factors over time can result in inter-annual changes in productivity in these systems (de Leeuw et al. 1990).

*Zostera capensi*s stem density in this study has been found to be approximately three times lower than that found by de Boer (2000) (Appendix A2). Although studies on other species in the genus suggest that that there may be a great degree of variability in *Z. capensis* stem density between different locations along its range, no other studies known to the author have quantified stem density of *Z. capensis* and it has been acknowledged that these aspects of *Z. capensis* have not been extensively studied (de Boer 2000). *Spartina maritima* stem density in this study was found to be between 3.5 times and 13 times lower than *S. maritima* stem density measurements found by Sánchez et al. (2001) in a narrow estuary in Ria de Betanzos, northwest Spain. Reasons explaining these high stem densities were not given. *Spartina alerniflora* stem density counts in Maryland, USA were more comparable to this study, ranging between 27 and 108 m^{-2} (Gleason et al. 1979).

Stem lengths of *Z. marina* have a great degree of variation and range between approximately 1.5 and 8 times longer than stem lengths found in this study (Appendix A3). No recordings of stem lengths of *S. maritima* were found in the literature, however low stem lengths of *S. alterniflora* in Parangua Bay in south-east Brazil were comparable to that of this study. (da Cunha Lana et al. 1991). Da Cunha Lana et al. (1991) explained that soil conditions, salinity stress as well as changing drainage dynamics have an effect on limiting the stem length of *S. alterniflora*.

The significantly higher stem length of *S. maritima* salt marsh than *Z. capensis* seagrass suggests that habitat complexity may be higher in *S. maritima* than in *Z. capensis*. This result is consistent with the above-ground biomass results between *Z. capensis* and *S. maritima* but is not in agreement with stem density. The use of plant structural elements in some studies have been found to be sufficient indicators of habitat complexity and have been found to be linked to faunal abundance. This has been found to be true for above-ground biomass (Heck and Wetstone 1977, Stoner 1982, Wyda et al. 2002), stem density (Vince et al. 1976, Hovel and Lipcius 2001, Wyda et al. 2002) and stem length (Gratwicke and Speight 2005, Gullström et al. 2008).

In accordance with the possible implications of biomass on habitat complexity as suggested by Heck and Wetstone (1977), Stoner and Lewis (1985) and Attrill et al. (2000), the higher biomass in *S. maritima* than *Z. capensis* may indicate that *S. maritima* provides greater food resources, greater living space, greater surface area and therefore better refuge from predation than *Z. capensis* and hence may function as a more important nursery habitat for *R. holubi* in the Bushmans Estuary. However, it has been argued that using above-ground biomass is not a good indicator of complexity as it does not directly measure plant shape, the amount of available living space and the nature of the spaces between plant structures, as well as complexity at a range of scales (McAbendroth et al. 2005). Hence, the assumption that biomass is an accurate indicator of complexity needs to be applied with caution. Below-ground biomass did not provide an indication of habitat complexity but did provide insight into the similar seasonal vegetation dynamics of *S. maritima* and *Z. capensis*.

Stem density and canopy height are considered to represent an aspect of plant structure, and they provide an indication of the arrangement of structural elements that comprise plant shape (Kovalenko et al. 2012). Habitat structure is likely to be linked to shelter for prey against predation and/or food organisms attached to and found in-between structures (Cocheret de la Morinière et al. 2004). Hence, the canopy height results suggest that *S. maritima* may provide marginally better protection from predators or increased food availability or a combination of the two than does *Z. capensis* and hence may function as a better nursery habitat for *R. holubi*. Conversely, the significantly greater stem density in *Z. capensis* than stem density in *S. maritima* suggests that *Z. capensis* may provide better protection from predators or increased food availability or a combination of the two than does *S. maritima* and hence suggests that *Z. capensis* may be a more valuable nursery habitat than *S. maritima* for *R. holubi*. However, it is critical to acknowledge that the measurement of stem density and canopy height does not account for the amount of cover provided for fauna, plant shape, and the nature of the spaces between plant structures (Warfe and Barmuta 2004, Warfe et al. 2008) and furthermore does not support between habitat comparisons (Kovalenko et al. 2012). It has also been suggested that stem density should not be viewed as a reliable indicator of habitat complexity in cases where stem density is highly variable within the same habitat type (Attrill et al. 2000), as was found in this study. Additionally, the quantification of plant structure does not distinguish between the scale dependent aspects of plant morphology pertinent to the use of the organism in question (Warfe et al. 2008).

Dimensionless indices

Total cover (C_t/A_t) results have shown that on average *Z. capensis* provides 0.28 m^{-2} more total cover per square meter than *S. maritima*. Few studies have assessed the total cover of plant species and hence it is difficult to draw comparisons to other species. Most C_t/A_t studies have assessed the effects of predation and survivorship of invertebrates and fish species in relation to artificial plant cover in laboratory and mecocosm experiments (e.g. Bartholomew 2002a; Bartholomew 2002b, Bartholomew and Shine 2008). Dowels used to closely replicate *S. alterniflora* stems were used by Bartholomew et al. (2000); the study revealed that C_t/A_t indices varied between different stem densities and were comparable to that of C_t/A_t of *S. maritima* and *Z. capensis* in this study. The total cover provided by plant species may therefore not only be dependent on the plant species under investigation, but also the stem density of that species (Bartholomew et al. 2000). The results of this index indicate that there is a greater two dimensional area available for use for protection from predators for *R. holubi* in *Z. capensis* than in *S. maritima*. This means that *R. holubi* inhabiting *Z. capensis* beds have more two-dimensional area in which to hide from view and have more available area to actively escape from view. Hence, according to this index, *Z. capensis* may provide better protection from predators than *S. maritima*.

Most studies that have implemented the ISI method have measured the ISI of individual plants (e.g. Dibble et al. 1997, Dibble and Thomaz 2006). However, the plant structure of both *S. maritima* and *Z. capensis* is simple when viewed individually and does not represent the true nature of complexity because stem density has not been taken into account. It was observed in this study that the complexity and the nature of the spaces between plant structures is dependent not only on individual structures, but additionally on how multiple plant structures occur in space, also taking into account the shape of those plant structures as they are influenced by tidal flow in the Bushmans Estuary. The high magnification photographs of whole individual structures used in this study are comparable to that of other studies that have analysed the ISI and fractal geometry of whole, individual plants. ISI has been found to vary: aquatic plant species such as *Eleocharis sphacelata, Triglochin procera* and *Myriophyllum spicatum* have a low degree of complexity in terms of the spaces between plant parts and thus low complexity values of less than five; aquatic plant species such as *Myriophyllum variifolium* on the other hand have high complexity values of 18 (Appendix 4). The ISI values of 2.43, 2.92 and 2.12 obtained for *S. maritima* in this study are comparable to other low complexity aquatic plant species. In contrast, *Z. capensis* ISI values of 12.91, 7.99 and 7.14 can be considered to be of intermediate complexity and are comparable to the ISI values of the aquatic plant species *Egeria densa, Zosterella dubia* and *Myriophyllum variifolium*

Many studies have assessed habitat complexity of vegetated habitats by analysing plant shape through the use of fractal geometry. The fractal dimensions of *S. maritima* and *Z. capensis* at high magnification (magnification=25cm^2), focusing on an individual macrophyte, indicate that these species have low to intermediate complexity compared to that of plant species in other studies (e.g. McAbendroth et al. 2005, Dibble and Thomaz 2009). This may be due to the relatively simple morphology of the two plant species in this study. The *S. maritima* fractal dimension at both magnifications was lower than most other plant species, while the fractal dimension of *Z. capensis* was more comparable to other plant species. *Zostera capensis* had a higher fractal dimension than *S. maritima* at both magnifications, suggesting that *Z. capensis* has a higher degree of complexity in terms of plant shape at two spatial scales. *Spartina maritima* and *Z. capensis* both had higher fractal dimensions at higher magnification than at intermediate magnification. Hence, according to the fractal dimension for both species, complexity is higher at higher magnifications. This trend is similar to that of some plant species in other studies (e.g. McAbendroth et al. 2005) (Appendix 5).

Stem density, ISI at three magnifications and fractal dimensions at two magnifications indicate that *Z. capensis* provides a habitat that offers a higher degree of complexity than *S. maritima*. Simple structural macrophyte components, such as stem density, biomass and stem length, are recognized as reliable indicators for within vegetated habitat comparisons (Kovalenko et al. 2012). However, it has been suggested that using this approach should not be viewed as dependable for between vegetated habitat comparisons of different macrophyte species (Kovalenko et al. 2012). The dimensionless indices consistently differentiated between *Z. capensis* and *S. maritima*. The areal cover provided by each habitat illustrated that there is greater living space in *Z. capensis* per square meter than *S. maritima*. The ISI index showed that the frequency and length of the spaces between plant structures was more complex in *Z. capensis* than in *S. maritima*. Furthermore, the higher fractal dimension in *Z. marina* than *S. maritima* at intermediate and high magnification has provided further insight into the differences in complexity between *Z. capensis* at different spatial scales. Each of the dimensionless indices used in this study provided evidence to suggest that complexity is higher in *Z. capensis* than in *S.maritima* and should be correlated to higher fish abundances (See Chapter Three). It is important to note that in the Bushmans Estuary *Z. capensis* is submerged during all tidal cycles and *S. maritima* is exposed at low and spring low tides. Therefore, the habitat complexity provided by *Z. capensis* is more available for use than in *S. maritima* in this study.

There has been little focus on what complexity indices are best for describing habitat complexity for fish in nursery areas. It can be assumed that fish in nursery areas need to be able to escape or to be hidden from view in order to avoid predation. Depending on their age, the length and size of fish vary within nursery habitats (Whitfield 1998). Therefore, it should be considered that different sized individuals may use different scales of complexity within nursery habitats (Dibble et al. 1997, Warfe et al. 2008), and complexity can vary at different scales (Dibble and Thomaz 2006). Dimensionless indices have been recommended as the most applicable indicators for comparing complexity of different plant species (Kovalenko et al. 2010). However C_t/A_t, ISI and fractals describe different aspects of complexity. Because C_t/A_t provides a measure of the total refuge area provided by different plant species, this index provides a direct measure of protection from predators. However, it has not been applied at different scales. The ISI method describes how space is broken up by plant structure at different scales (Dibble et al. 1997). As fish seeking refuge need to be hidden from view to avoid detection, a lower length of spaces and a higher frequency of spaces will possibly hide fish shape and reduce rates of predation. Combining ISI of multiple plants accurately reflects habitat structure and therefore accounts for complexity over a greater area. Hence it is an accurate indicator of refugia and therefore nursery role. However, it is important to note that replicating images of habitat structure is important in order to account for heterogeneity within habitats. Using fractals may not necessarily be of more relevance than ISI and C_t/A_t for assessing complexity of macrophytes for fauna (Kovalenko et al. 2012) but may be more powerful when used to qualitatively assess complexity of a greater variety of habitat types such as rocky habitats and coral reefs (Tokeshi and Arakaki 2012).

Conclusion

Estuarine habitats of high habitat complexity such as seagrasses, salt marshes and coral reefs in shallow marine environments have high ecological value because they function as important nursery areas for fauna such as macroinvertebrates and juvenile fish species (Whitfield 1989a, Bell et al. 2001)**.**

This study shows that using multiple approaches for assessing habitat complexity can yield different results, underlining the importance of using multiple approaches for assessing complexity. However, the relatively consistent results of the dimensionless indices suggest that *Z. capensis* exhibits an overall greater degree of complexity than *S. maritima,* and hence it can be expected that *R. holubi* abundance is likely to be higher in *Z. capensis* seagrass than in *S. maritima* salt marsh. Critically, dimensionless indices that analyse complexity at different spatial scales enable a more complete analysis of habitat complexity as they allow for direct comparisons between habitat types. Furthermore, using multiple complexity indices in habitat complexity studies provides a thorough approach to habitat complexity analyses (Kovalenko et al. 2012).

Although there is much evidence to suggest that macroinvertebrates use specific shapes provided by plants as refuge from predators, there is little empirical evidence to suggest that fish utilize specific aspects of plant structure for protection from predators, or whether total cover is a more appropriate index. More research is needed to devise techniques for analysing complexity at the correct scale for fish species.

In the next chapter, I will attempt to quantify the relative abundance of *R. holubi* in the dominant habitats in the Bushmans Estuary to ascertain if *R. holubi* abundance is related to habitat complexity.

CHAPTER FOUR

DETERMINING THE USE OF HABITATS AS NURSERY AREAS FOR *R. HOLUBI*

INTRODUCTION

Rhabdosargus holubi is considered to be entirely dependent on estuaries for at least the first year of life (Whitfield 1994). Although estuaries function as critical nursery areas for estuarine-dependent marine species, few studies have quantitatively assessed the nursery value of specific habitat types within estuaries for juvenile fish species. Beck et al. (2001) suggest that any habitat that makes a greater than average contribution to the recruitment of adults should be considered a nursery habitat. As such, not all juvenile habitats are nurseries. Good nursery habitats provide protection from predators, and thus allow for higher survival, and an abundance of food resources, allowing for high specific growth rates (Beck et al. 2001). Although density is only one of the four factors that Beck et al. (2001) suggest must be considered to determine whether a habitat serves as a nursery, most studies quantifying the nursery value of habitats, specifically seagrass, salt marsh and un-vegetated habitats, have used higher densities of fish in different habitats as an indication of nursery value (e.g., Minello 1999, Minello et al 2003, Heck et al. 2003), with density regarded as an important indicator of recruitment and emigration (Minello 1999). Higher densities of juvenile fish are often found in structurally complex habitats, such as salt marshes, mangroves and seagrasses. In the Bushmans Estuary vegetated habitats are more structurally complex than non-vegetated habitats, and *Zostera capensis* seagrass beds provide more habitat complexity than do *S. maritima* dominated salt marsh beds (Chapter Two).

Although *R. holubi* occurs throughout shallow estuarine habitats within estuaries (Becker et al. 2010, 2012, Sheppard et al. 2012) it is regarded as a vegetation-associated species (Sheppard et al. 2012). More specifically, *R. holubi* is known to be associated with submerged macrophyte beds such as *Z. capensis* (Hanekom and Baird 1984). In the Kromme Estuary *R. holubi* was found to be significantly more abundant in *Z. capensis* than in non-vegetated areas (Hanekom and Baird 1984).

It is therefore hypothesized that the relative abundance of *R. holubi* in the Bushmans Estuary will be higher in vegetated habitats than in non-vegetated habitats and highest in *Z. capensis* seagrass beds. *Rhabdosargus holubi* should also exhibit behaviour associated with a high degree of habitat use such as slow meandering in structured habitats and behaviour associated with a low degree of habitat use such as rapid swimming in unstructured habitats.

Appropriate gear selection that allows for quantitative sampling and is suitable for use across different habitat types is a critical requirement for studies involving between habitat density comparisons in shallow estuarine habitats (Rozas and Minello 1997). Rozas and Minello (1997) reviewed the suitability of sampling designs commonly used for estimating the density of nekton associated with shallow estuarine habitats (see Table 4.1). Few netting gears are suitable for many or all habitat types as they, for example, have varying catch efficiencies in different habitat types. Those that are recommended for sampling in multiple habitat types, such as drop samplers, have other clear disadvantages such as being expensive to make, challenging to operate in the field and have small sample unit areas (Rozas and Minello 1997).

Table 4.1: Gear use and recommendations for quantitative sampling of small nekton in shallow estuarine habitats. (Rozas and Minello 1997). R = highly recommended; C = conditionally recommended (only under appropriate circumstances or gear is modified); N= not recommended.

Gear Type	**Non-vegetated sub-tidal**	**Non-vegetated inter-tidal**	**Seagrass or SAV**	**Tidal marsh**	**Oyster reef**
Encircling or block net	C	C	C	N	C
Purse seine	C	C	N	N	N
Flume weir	N	N	N	R	N
Drop net	N	N	C	C	C
Throw trap	R	R	R	C	C
Drop sampler	R	R	R	R	R
Pop net	C	C	R	N	C
Bottomless lift net	N	N	C	R	C

Recent studies in estuaries have quantified the relative abundance of fish species by deploying underwater video cameras for the visual recording of fish (Watson et al. 2009, Becker et al. 2010, 2012, Harvey et al. 2012, Smith et al. 2012). The use of underwater video cameras has become a useful approach for effectively measuring the relative abundance of fish in multiple habitat types (Becker et al. 2010, 2012) and has the additional benefit of also observing fish behaviour (Becker et al. 2011).

Aims and objectives

The aim of this chapter was to determine the use of habitats in the Bushmans Estuary as nurseries for *R. holubi*. The objective was to assess the relative abundance and behaviour of individuals within the dominant habitat types found in the estuary. The relative abundance of *R. holubi* was used as an indicator of nursery value for each habitat type as it reflects recruitment, mortality and emigration.

MATERIALS AND METHODS

Pilot study

A throw trap was designed for the sampling of juvenile *R. holubi* in multiple habitat types. Aluminium steel rods were used to construct a 1 m^2 square frame, 1 m wide and 0.5 m in height. A 5 mm nylon mesh net was attached to the sides of the enclosure as outlined by Kushlan (1981). The corners of the frame were reinforced with short aluminium rods and iron sinkers were attached to the bottom frame to increase the rate at which the throw trap sank during operation. The efficacy of the 1 m^2 throw trap was tested in seagrass, salt marsh and sand flat habitats in the Bushmans Estuary. The throw trap was thrown 15 times in each habitat by two persons standing on either side of the trap. No fish were captured during the pilot study and hence the 1 m^2 throw was deemed ineffectual.

Field sampling

GoPro Hero 3 Silver underwater video cameras were subsequently chosen for quantifying *R. holubi* abundance in multiple habitats. Underwater videos were filmed between November 2013 and September 2014 during spring high tide cycles. Cameras were deployed concurrently in each habitat type (seagrass, salt marsh and sand flats). There were three sites in each habitat type, giving a total of nine sites (Fig. 4.1). Sites were all in the lower reaches of the estuary, below the R72 road bridge, as turbidity was too high for filming above the bridge. The cameras were attached to 1.5 m long white perspex poles marked with depth measurements. The cameras were attached with cable ties to the poles and inserted vertically into the sediment, resulting in the camera positioned to capture a 180° view horizontal to the estuary floor and a field of view of the entire vertical length of the water column. The three cameras were deployed concurrently in each habitat type for a total of 45 minutes before being rotated between each of the three spatially replicated sites, resulting in 2 h 15 min of filming per habitat during each spring high tide cycle.

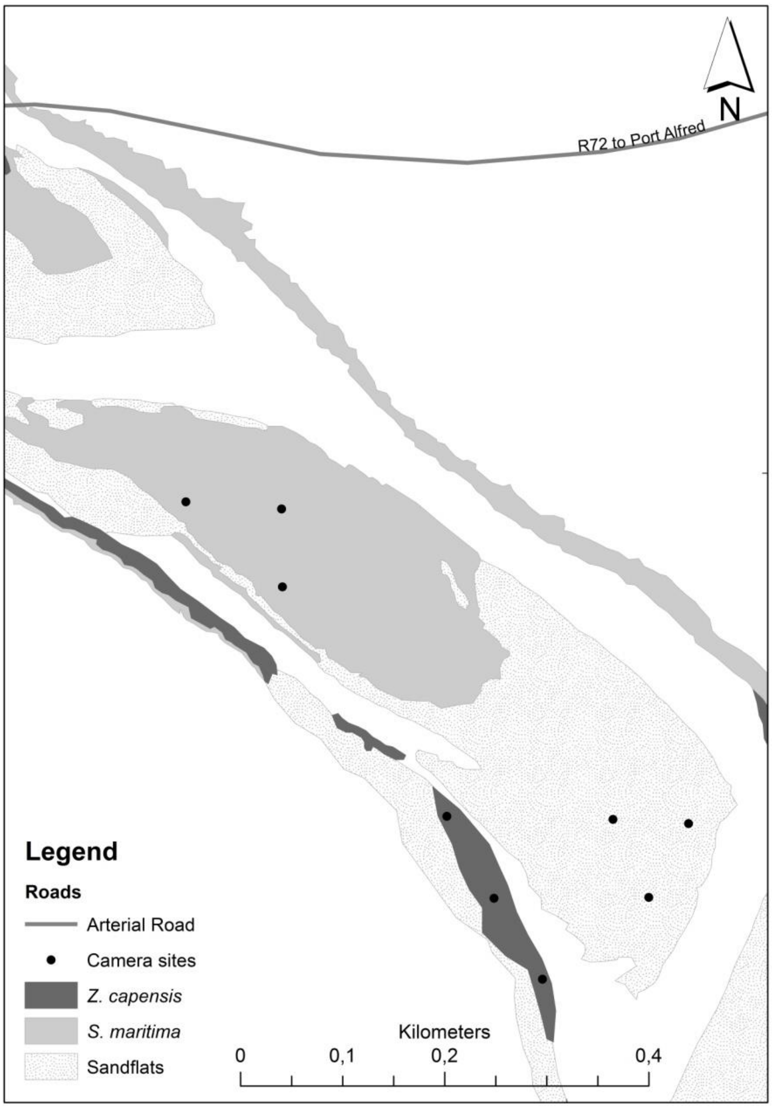

Figure 4.1: Map of the lower reaches of the Bushmans Estuary, showing the nine camera sites selected for underwater video camera filming in seagrass, salt marsh and sand flat habitat types

In the vegetated habitats, the cameras were positioned in open patches or along channels within the macrophyte beds and on the vegetation edge with the camera's field of view parallel to the vegetation edge. Forty-two hours of footage were obtained in a total of 56 deployments during the 11 month sampling period. The selected habitat types were greater than 150 m apart and hence deemed independent. Sites in each habitat were placed at a minimum of 30 m apart to account for heterogeneity within habitats.

The chosen sites in all habitats were equal in water depth to negate any tidal effects on *R. holubi* distribution. During the 2 h 15 min of filming, water depth measurements were taken at 1 hour intervals. Water depth ranged between 0.7 and 1.1 m, and the rate of change in water depth during the spring high tide cycle was approximately equal between habitats (±10 cm). Upon the deployment of cameras, a five minute acclimation period was allowed before footage was included in analysis. Water samples were taken every 45 minutes at each of the sites. Turbidity was measured subsequently in the laboratory using a Hanna turbidity meter to ensure that visibility was adequate for filming and that visibility was similar between deployments. Turbidity measurements below 4 NTU were required to ensure visibility of approximately 3.5 m (Becker et al. 2010). Filming during turbidities above 6 NTU were subsequently abandoned. *Rhabdosargus holubi* video footage taken throughout the year remained appropriate as this species is a serial spawner, with recruitment recorded throughout the year in Eastern Cape estuaries (Blaber 1973).

Video footage analysis

Video camera footage was viewed on a laptop computer and analysed using the video editing program Adobe Premiere Pro CC. The MaxN approach was used to quantify the relative abundance of *R. holubi* in each habitat type. Each 45 minute deployment was divided into three 15 minute ‘slots’. The maximum number of *R. holubi* individuals (MaxN) observed in a single frame during each 15 minute slot was counted. The MaxN was taken for each of the three slots in the 45 minute deployments at each site, and a mean MaxN calculated per deployment.

The behaviour of each *R. holubi* individual viewed in the footage was assigned to one of the four categories used by Becker et al. (2010), which consisted of 1) slow meandering, involving an individual slowly moving past the field of view 2) stop start, where an individual swims into the field of view, stops and then moves on 3) rapid swimming, where an individual rapidly swims past the field of view and 4) feeding, in which an individual is clearly feeding on macrophytes, in the water column or off the benthos. Slow meandering and feeding is viewed as behaviour associated with a high degree of habitat use, stop-start an intermediate degree of habitat use, and rapid swimming a low degree of habitat use. Individuals predominantly showed one behaviour type. However, in cases where multiple behaviour types were displayed, the behaviour that lasted for the longest period of time was included in the analysis.

Data analysis

Differences in turbidity between habitats and between sites within habitats were tested using a two factor nested ANOVA. Each habitat (sand flats, salt marsh and seagrass) was used as a fixed factor, and sites were nested in each habitat type as three random categories. Data were square root transformed and a Cochran's C test was used to test the assumption of the homogeneity of the variances. Differences in relative abundance (MaxN) between habitats and sites within habitats were also tested using a two factor nested ANOVA. After multiple data transformations, data did not meet the assumption of the homogeneity of the variances. However, sizeable ANOVAs are known to be resilient to deviances from this assumption (Quinn and Keough 2002, Becker et al. 2012). Each of the four behaviours exhibited by *R. holubi* in each habitat was evaluated using Chi-squared contingency tests. The observations in each behaviour category were pooled for all sites within each habitat.

Complexity indices from the previous chapter were used to examine the relationship between habitat complexity and relative abundance (MaxN) of *R. holubi*. Mean MaxN for each of the sites in each habitat type was compared to the stem density of each transect in each habitat (averaged across each of the four seasons), and the average ISI and fractal dimensions for each of the three images used to calculate complexity in the seagrass and salt marsh habitats. It was assumed that sand flats had no complexity.

RESULTS

Turbidity across all sites ranged between 1.9 and 3.9 NTU. There was no significant difference in turbidity between habitat types ($F_{2,\ 49}$=1.32, p>0.05) or between sites within habitat types ($F_{6,\ 49}$= 0.46, p>0.05).

Seagrass had higher mean MaxN values than salt marsh and sand flats across all sites (Fig.4.2). There was a significant difference in mean MaxN values between habitat types ($F_{2,\ 159}$=21.76, p<0.0001) but no significant difference in mean MaxN values between sites within habitats ($F_{6,\ 159}$=0.14, p>0.05). Tukey post-hoc analysis revealed that mean MaxN was significantly higher in seagrass than in salt marsh and sand flats. There was no significant difference in mean MaxN between salt marsh and sand flats (d.f. =159, p<0.001).

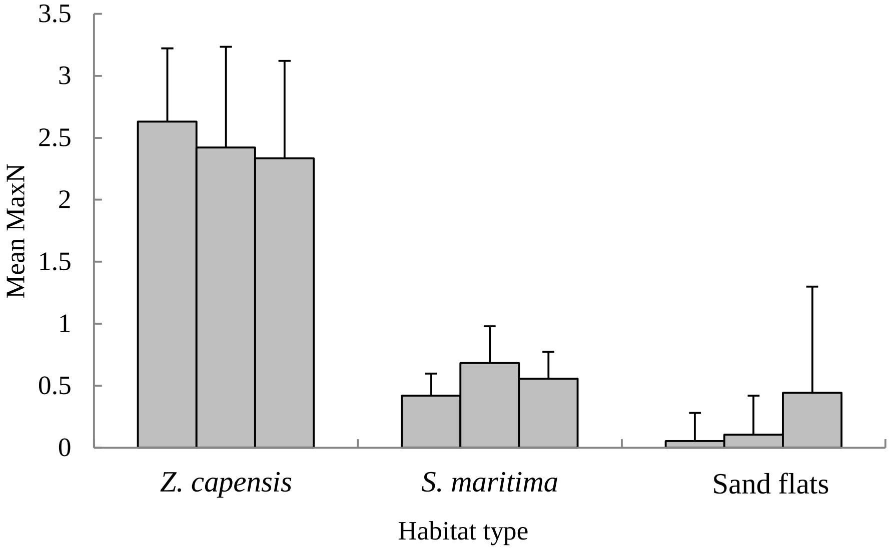

Figure 4.2. The mean MaxN (±SE) of *R. holubi* pooled across all deployments at within each of the three habitat types (each bar indicates one of three sites in each habitat type).

The Chi-squared contingency tests revealed that there was a significant difference in the behaviour of *R. holubi* between each habitat (χ^2=50.11, d.f. =6, $p<0.05$). Thus, the null hypothesis that there is no difference in behaviour between each habitat was rejected. In seagrass, slow meandering was the dominant behaviour exhibited by *R. holubi.* Out of 256 observations, slow meandering was observed on 160 occasions (62.5%) (Fig. 4.3), while start stop was observed on 26 occasions (10.1%), rapid swimming on 59 occasions (23.04%) and feeding on 11 occasions (4.2%). Slow meandering was the most frequent behaviour observed in salt marsh. Out of a total of 41 observations, slow meandering was observed on 21 occasions (51%), start stop on 15 occasions (36.9%), rapid swimming on five occasions (21.2%) and feeding on zero occasions. In the sand flats, rapid swimming was the dominant behaviour exhibited. Out of 15 observations, slow meandering was observed on two occasions (13.3%), start stop on zero occasions, rapid swimming on 13 occasions (86.6%) and feeding on zero occasions .

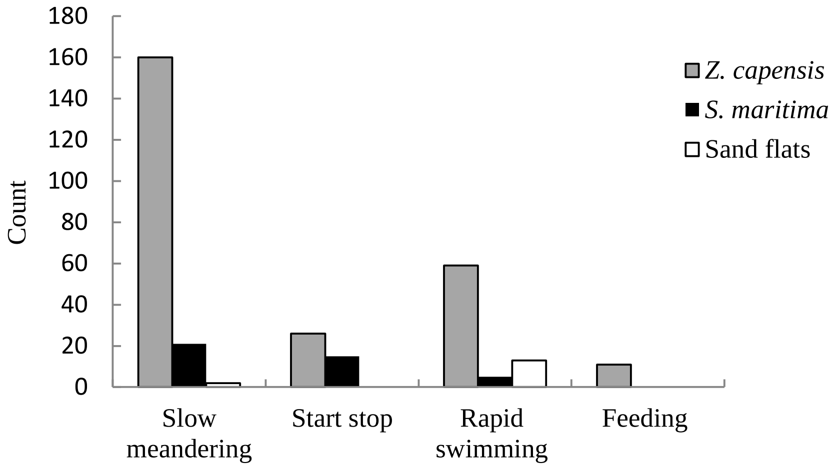

Figure 4.3: The total count of *R. holubi* viewed exhibiting each of the four behaviour categories in each of the three habitats within the lower reaches of the Bushmans Estuary between November 2013 and September 2014

Mean MaxN was highest in the three seagrass sites (2.6, 2.4 and 2.3) and lowest in the three sand flat sites (0.05, 0.1 and 0.44). Abundance (represented by mean MaxN) of *R. holubi* increased with increasing stem density (Fig. 4.4), interstitial spatial index (ISI) (Fig. 4.5a), total cover per habitat area (C_tA_t) (Fig. 4.5b), and fractal dimension at intermediate and high magnifications (Fig. 4.5c and Fig. 4.5d). All indices of habitat complexity were higher in seagrass sites and lowest in the sand flat sites.

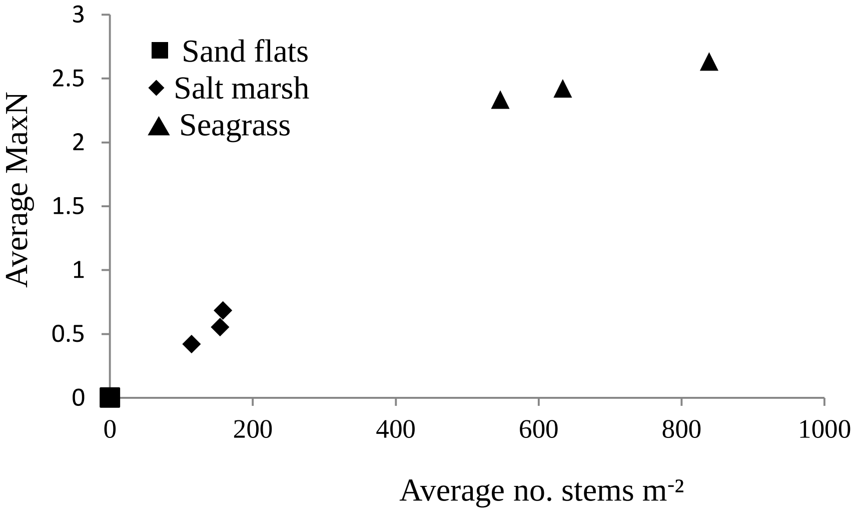

Figure 4.4: The relationship between stem density and relative abundance of *R. holubi* in each habitat type.

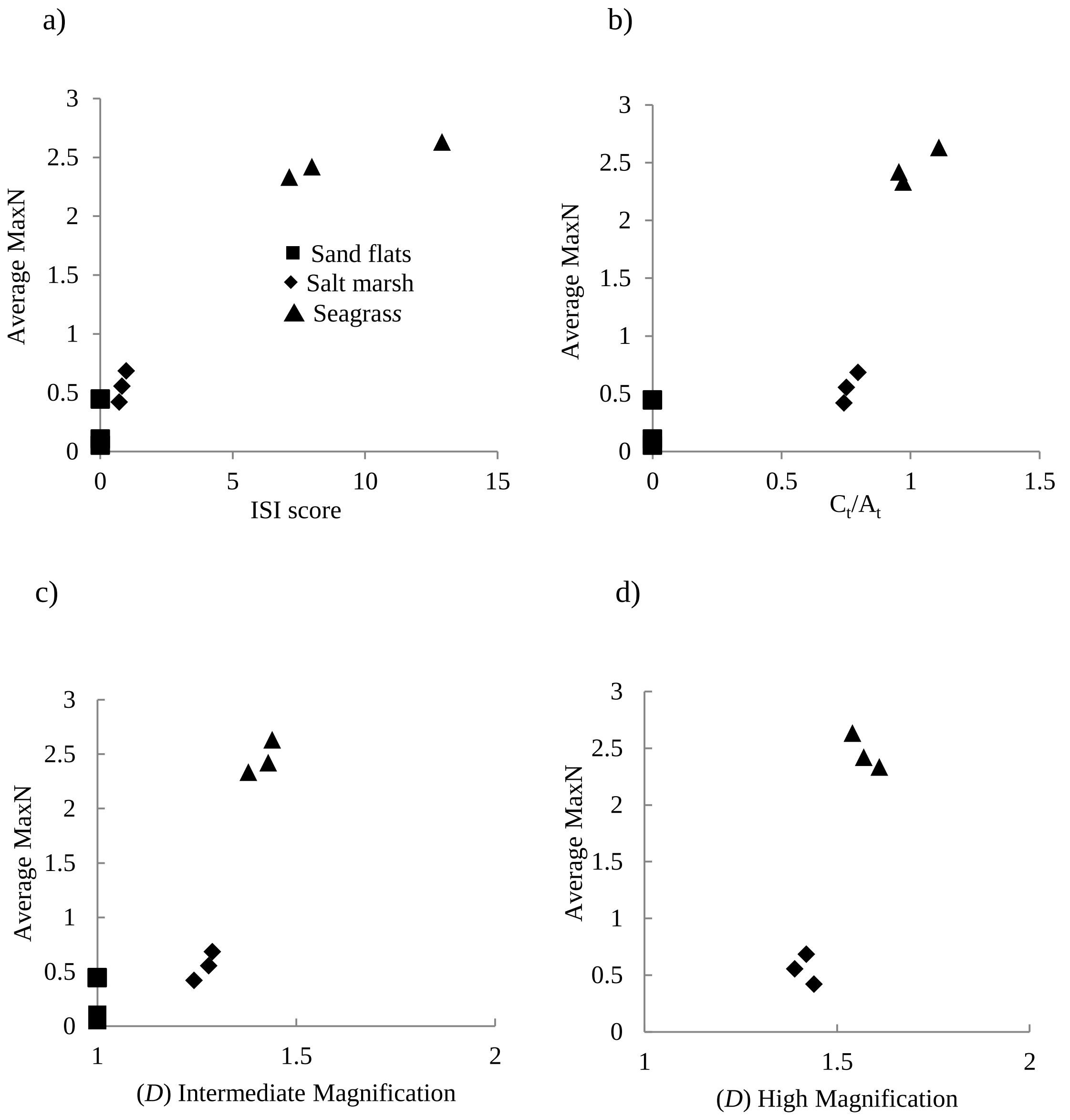

Figure 4.5 a) The relationship between the ISI index scores and relative abundance of *R. holubi*; b) total macrophyte cover (C_t/A_t) and relative abundance of *R. holubi*; c) the fractal dimension at intermediate magnification and relative abundance of *R. holubi*; d) fractal dimension at high magnification and relative abundance of *R. holubi* in each habitat type

DISCUSSION

The significantly higher abundance of *R. holubi* in seagrass compared to salt marsh and the sand flats in this study has provided evidence to support the theory that not only are juvenile *R. holubi* a vegetation-associated species, but also a species that prefers seagrass to salt marsh. The higher incidence of slow meandering observed in seagrass sites also indicates behaviour associated with a high degree of habitat use.

The use of underwater video cameras and the MaxN approach proved to be a useful method for quantifying the relative abundance of *R. holubi* in vegetated and un-vegetated habitats. The underwater video cameras were easily deployed in shallow estuarine habitat types, the abundance of *R. holubi* was quantified over fairly short time periods, and the underwater video cameras proved to be especially beneficial when studying fish in small, fragmented habitats that are problematic when using traditional netting gear (Becker et al. 2012). There were, however, certain limitations to using the underwater video cameras. The structure and colour of the white poles used for the attachment of the underwater video cameras may have been easily identified as a foreign object by *R. holubi* and this may have influenced the abundance and/or behaviour of *R. holubi*. In future, it may be advisable that the poles be painted a darker colour (black or dark green) in order to negate this possible effect. Underwater cameras were limited to the edges of submerged aquatic vegetation as deploying cameras in densely vegetated patches obstructs the field of view, and deploying underwater video cameras on the edge of vegetated habitats prevents the cameras from filming an entire field of view (Becker et al. 2012). Another drawback to using this method is that the MaxN relative abundance measurements cannot be directly compared to studies that have measured fish density using equipment such as traditional netting gear.

The results of this study are in accordance with several other studies. Whitfield et al. (1989b) compared ichthyofaunal assemblages in seagrass beds and un-vegetated areas in the Knysna and Swartvlei estuaries. *Rhabdosargus holubi* were more abundant in seagrass beds compared to non-vegetated areas in both estuaries, with *R. holubi* composing 11% and 10% of the fish caught in dense and sparse seagrass beds of the Knysna Estuary, compared with only 3% of the fish caught in unvegetated areas. Similarly in the Swartvlei estuary, *R. holubi* comprised 6% and 15% of the fish caught in dense and sparse seagrass beds and only 0.11% of the fish in non-seagrass areas (Whitfield 1989b). Paterson (1998) investigated the ichthyofauna of three creeks associated with *Spartina maritima* and *Sarcocornia perennis* dominated salt marsh flats in the Kariega Estuary and compared the ichthyofauna to that of neighbouring *Z. capensis* seagrass beds. *Rhabdosargus holubi* densities were found to be significantly higher in seagrass than the salt marsh creeks.

Whitfield (1986) also recorded a gradual decrease in the catch per unit effort of *R. holubi* over a 4 year period following the senescence of *P. pectinatus* seagrass, the disappearance of filamentous algal mats and the subsequent establishment of sandy habitats in the Swartvlei Estuary. Similarly, Sheppard et al. (2012) recorded a decrease in the contribution of *R. holubi* to the total catch per unit effort during the macrophyte senescent phase of *Ruppia cirrhosa* and *Potamogeton pectinatus* seagrass in the East Kleinemonde Estuary. The loss of seagrass habitats and the subsequent decline in juvenile fish abundance indicate the importance of seagrass habitats for this species (Heck et al. 2003).

In a review of the nursery role of seagrass habitats, Heck et al. (2003) found little evidence of differences in abundance, growth and survival of species in various types of structurally complex habitats. They concluded that vegetated and other structurally complex habitats such as reefs provide protection from predation, which may allow for more time for feeding and thus greater growth rates than unstructured habitat, as well as more substrate for food to grow, again influencing growth rates. Minello et al. (2003) in a review of the nursery function of salt marsh habitats also found that survival in salt marshes was significantly higher than survival in open water, significantly lower than in oyster reef/cobble and not significantly different from seagrass. In this study, the abundance of *R. holubi* was significantly greater in seagrass sites than in either salt marsh sites (structurally complex habitat) or un-vegetated sand flats. Differences in the abundance of *R. holubi* in the two vegetated habitats may be due to differences in habitat complexity. Five of the seven complexity indices used in Chapter Two indicate that seagrass is a more complex habitat than salt marsh and the un-vegetated sand flats.

Becker et al. (2012) used video cameras to assess the influence of depth changes associated with tides along seagrass bed edges in the Bushmans Estuary. They found that there was no significant difference in the relative abundance of *R. holubi* between seagrass edges and sand sites in the lower reaches of the Bushmans Estuary across tidal cycles. Although these findings are contrary to those of this study, this discrepancy may be explained by the difference in the distance between sand and seagrass sites in each study. Becker et al. (2012) positioned the remote underwater video cameras at sand sites directly between and within relatively close proximity to seagrass beds (approximately 50 m apart). In this study the selected sand sites were greater than 150 m apart from the closest seagrass bed and were in discrete zones within the sand flats. Consequently, we may have recorded fewer *R. holubi* individuals moving between and within close proximity to seagrass beds.

The lower abundance of *R. holubi* in salt marsh may indicate that *R. holubi* respond to different aspects of structure, as has been indicated between reef and reed habitats in the East Kleinemonde Estuary (Becker et al. 2010). Many studies have suggested that habitats of higher complexity can lead to a higher degree of protection from predators and may lead to increased survival (Bartholomew et al. 2000, Minello et al. 2003, Warfe and Barmuta 2004).

Using underwater video footage allowed for estimations of the abundance of individuals in different habitats as well as examining their behaviour. The higher incidence of slow meandering observed in seagrass sites indicates behaviour associated with a high degree of habitat use. Protection from predation provided by structurally complex habitats may allow more time for feeding and thus greater growth rates (Heck et al. 2003. Feeding was also only observed in seagrass sites. According to Heck et al. (2003) studies assessing the nursery function of habitats must focus not only on abundance but also on survival of individuals. Survival is influenced by both protection from predation and an abundance of food resources.

In the salt marsh and sand flat sites there was a much higher degree of start stop and rapid swimming behaviours, which indicates a lower degree of habitat use, with individuals moving through these habitats. Becker et al. (2012) also found that slow meandering was the dominant behaviour of *R. holubi* in seagrass edges and rapid swimming the dominant behaviour in sand sites, supporting the findings of this study.

As indicated by the behaviour of individuals in the different habitats the structurally complex seagrass habitats in the Bushmans Estuary may provide nursery habitat to *R. holubi* through both protection from predation and an abundance of food resources (which may in part be related to more structure available for food to grow). Investigating the diet of *R. holubi* in the different habitat types will provide further understanding of the relative importance of habitat types as nursery areas for *R. holubi*.

Conclusion

The findings of this study have provided evidence to support the hypothesis (i) that *R. holubi* density is higher in vegetated habitats than in non-vegetated habitats and highest in *Zostera capensis* seagrass beds and (ii) that *R. holubi* exhibits behaviour associated with a high degree of habitat use in structured habitats and behaviour associated with a low degree of habitat use in unstructured habitats. The findings of this chapter also revealed that the relative abundance and behaviour of *R. holubi* were aligned with the habitat complexity findings in Chapter Two.

In the next chapter I will attempt to gain more insight into the nursery role of habitat types in the Bushmans Estuary by using stomach content analysis and diatom composition analysis to understand more about the feeding habitats of *R. holubi*.

CHAPTER FIVE

USING STOMACH CONTENT ANALYSIS AND DIETARY DIATOM COMPOSITION TO IDENTIFY THE ESTUARINE FEEDING HABITATS OF JUVENILE *RHABDOSARGUS HOLUBI*

INTRODUCTION

An abundance of quality food resources is critical for high growth rates for juvenile fishes (Gibson 1994), and habitats that provide this are critical to facilitate the recruitment of juveniles into adult populations (Beck et al. 2001). Hence, an understanding of the food resources used by fish species is crucial for quantifying the nursery value of different habitat types for juvenile fishes within estuaries.

Rhabdosargus holubi is omnivorous and plant matter consisting of filamentous and submerged aquatic macrophytes such as *Zostera capensis, Ruppia spiralis* and *Potamogeton pectinatus* have been found to form a dominant component of the diet of juveniles (<30 mm SL) (Blaber 1973, Whitfield 1984). Invertebrates, predominantly belonging to the subphylum Crustacea have been found to constitute the remainder of the diet (de Wet and Marais 1990). While there have been several dietary studies conducted on *R. holubi* (e.g. Blaber 1973, Whitfield 1984, Whitfield 1988, de Wet and Marais 1990, Schlacher and Wooldridge 1996), all of these grouped the fish captured in different habitats, and therefore there is limited information available on differences in diet between habitat types.

Although submerged aquatic macrophytes are a dominant component of the diet of *R. holubi*, this species lacks cellulase in its digestive system. It has been suggested that *R. holubi* mostly digest the epiphytic diatomaceous layer and other algae found on the leaves, with the macrophytes passing through the gut undigested (Blaber 1974, de Wet and Marais 1990). Sheppard et al. (2012) conducted an isotope study to determine the importance of macrophytes as a primary dietary source for selected fish species in the East and West Kleinemonde estuaries. They found that although seagrass was present in the stomachs of *R. holubi*, epiphytic algae were the most important food source for *R. holubi*.

Different diatom assemblages colonize the seston and the surface of plants, rocks and sediment (Taylor et al. 2007). Diatoms are sensitive to environmental conditions and hence the species composition of diatoms occurring in various habitat types can vary spatially and seasonally within estuaries (Stoermer and Smol 1999). Herbivorous fish species that ingest aquatic plants and filamentous algae are likely to consume the diatomaceous layer occurring on the leaves of aquatic plants and filamentous algae, and hence, the diatom assemblage in the fish stomachs is likely to reflect the diatom assemblage occurring on plant matter in the environment. In estuaries, a lot of the research on diatoms has been focused on their use as indicators of environmental conditions and environmental change (e.g., Stoermer and Smol 1999). Few studies have investigated the use of diatoms as indicators of feeding habitat in ecological studies. Tall et al. (2006) assessed resource partitioning within an invertebrate grazer guild by comparing the diatom assemblage occurring on a periphyton mat (*Fontinalis dalecarlica*) and the diatom assemblages ingested by invertebrates in a small Quebec stream. Very few, if any studies, have focused on the use of diatoms as indicators of feeding habitat in fishes.

Aims and objectives

The aim of this chapter was to evaluate the nursery value of four dominant estuarine habitats of the Bushmans Estuary for *R. holubi* by analysing the dietary composition of individuals caught in the four different habitats and to compare the diatom assemblage in seagrass with that in the diet to determine whether feeding habitat can be inferred from dietary diatom composition. The objectives were to: 1) describe the diet of *R. holubi* caught in four estuarine habitats, namely *Z. capensis* seagrass, *S. maritima* salt marsh, sand flats and mudflats; 2) identify the dietary items that are assimilated by *R. holubi*; 3) infer which habitat types *R. holubi* are likely to feed in; 4) describe the diatom assemblage in the *Z. capensis* seagrass; and 5) describe the diatom composition in the diet of *R. holubi*.

MATERIALS AND METHODS

General stomach contents analysis

The contents of 480 juvenile *R. holubi* foreguts were collected in the spring of 2013 and the summer, autumn and winter of 2014. Thirty juvenile *R. holubi* individuals between 4cm and 15cm SL were caught in the sand flats, mud flats, salt marsh and seagrass habitats each season using a 20 m seine net (see Fig. 5.1). Fish were euthanized by placing them on ice for 30 minutes, and the effect of post-capture digestion was reduced by injecting ethanol (95%) into the body cavity using a hypodermic needle.

In the laboratory, the contents of each stomach were emptied, spread into a petri dish and mixed with a predetermined amount of fifteen millilitres of 95% ethanol. The contents were identified to the lowest possible taxon using a dissecting microscope at a magnification of between 10× and 40×. The percent volume (%V) for each food item was estimated using the methods described by Potts and Khumalo (2005). The frequency of occurrence was recorded as the percent of stomachs containing a specific food item (%F).

A ranking index method (RI) was used to compare the relative importance of prey items of *R. holubi* from different habitat types. This was calculated by multiplying the percentage volume by the frequency of occurrence (Berg 1979) and expressed as a percentage.

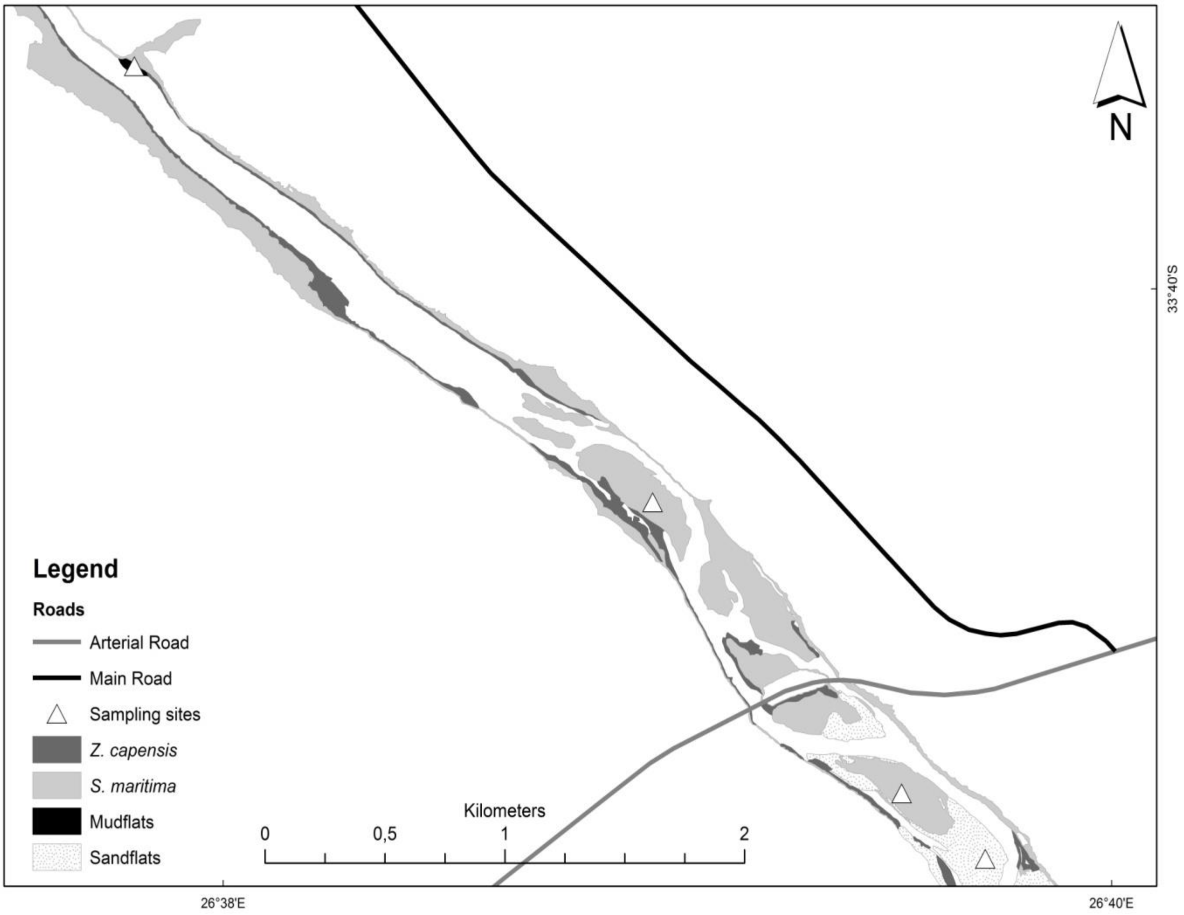

Figure 5.1: Map of the lower reaches of the Bushmans Estuary showing the different habitat sites selected for capturing *R. holubi* in each season

Diatom composition in the seagrass habitat

Zostera capensis leaves were collected in nine equally spaced locations in the seagrass bed (Fig. 5.1) and placed into small plastic bags. Epiphytic diatoms were then scraped off the entire length of each seagrass leaf using a blunt blade as recommended by Dauby and Poulicek (1995) and preserved and stained in Lugol's iodine.

Each diatom sample was observed using an inverted compound microscope at a magnification of 400× and the first 200 diatoms observed were identified to the lowest possible taxon using the keys developed by Taylor et al. (2007).

Diatom composition in the diet

The stomach contents of eight *R. holubi* from each habitat type (mud flats, sand flats, seagrass and salt marsh) from each season were preserved and stained in Lugol's iodine solution. This mixture was stirred thoroughly; 15 ml was put in a petri dish and the first 200 diatoms were identified using the methods described above.

Statistical analyses

General diet data were square-root transformed to ensure normality. Analysis of similarity (ANOSIM) was performed using PAST version 2.17C to describe the differences in the diets of *R. holubi* between sites and seasons

The similarity between the diatom composition in the diet of *R. holubi* caught in each habitat type and the diatom composition occurring in the seagrass habitat was compared using a Bray–Curtis similarity analysis for each season. Classification and ordination were used to plot the results. The composition data were square-root transformed to ensure normality.

RESULTS

General stomach contents analysis

Of the 480 foreguts examined, 52 (10.8%) were empty and subsequently excluded from the analysis (Table 5.1). Stomach contents, in terms of percentage volume (%V), predominantly consisted of plant matter, including red filamentous algae (40%) and seagrass (33.5%) (Table 5.2). Invertebrates contributed a considerably lower proportion to the total diet, with sesarmid crabs (4.6%), Assiminea spp. (3.5%) and anomura crabs (2.9%) the dominant prey items. In terms of frequency of occurrence (%F), red filamentous algae (58.8%) and seagrass (54.8%) were the most frequent food items recorded in the stomachs of *R. holubi,* followed by gastropoda (17.5%) and amphipoda (8.3%). Overall, as indicated by the percentage ranking index (RI), red filamentous algae (52.3%) and seagrass (40.9%) appeared to be the most important dietary components of *R. holubi* followed by gastropods (1.4%) and brachyura (0.4%).

Table 5.1: Description of the stomach fullness (% empty) of *R. holubi* stomachs based on the presence/absence of food in the foregut examined across seasons (spring 2013, summer, autumn and winter 2014) and habitat types

Season	**Mud flat**	**Sand flat**	**Seagrass**	**Salt marsh**	**Combined**
Spring	6.7	6.7	6.7	23.3	10.8
Summer	10	16.7	10	10	11.7
Autumn	10	10	6.7	3.3	7.5
Winter	6.7	23.3	10	13.3	13.3
Combined	8.3	14.2	12.5	8.3	

When comparing the diets by habitat, red filamentous algae dominated the diet of fish captured in mud flats (%V = 57.8; %F = 82.5), sand flats (%V = 40.1; %F = 53.3) and seagrass beds (%V = 45.3; %F = 64.2%). However, red filamentous algae were less dominant (%V = 16.3; %F = 35.0) in the diets of fish captured in the salt marsh habitat. The diet of fish captured in the salt marsh habitat was dominated by seagrass (%V = 56.6, %F =72.5). Seagrass was less important in fish captured in the mud flats (%V = 22.1, %F =50.0), sand flats (%V = 27.0, %F = 51.7) and seagrass (%V = 29.4, %F = 45.0).

Amphipoda (%F = 20; %V = 2.8) and copepods (%V = 4.96%, %F = 10.0%) were more important in the diets of fish captured in the mud flats compared with the other habitats, where they were almost absent (Table 4.2). Mysid shrimps were present in the diet of fish captured in the mudflats (%V = 0.9) and seagrass (%V = 1.3%), but absent in the diet of fish captured in the other habitats. Brachyura were more important in the diet of fish captured in the seagrass (%V = 7.8, %F = 12.5) and salt marsh (%V = 5.9, %F = 10.0) habitats than in the sand flats (%V = 2.5%, %F = 1.7) and mud flats (%V = 2.0, %F = 6.7). Bryozoans were an important dietary component (%V = 12.1; %F = 18.3) in the sand flats compared with the other habitats (%V < 0.1; %F = 0 – 0.8%). Gastropoda were found consistently (%F > 15%) in the diets of *R. holubi*, regardless of habitat (Table 4.2).

Table 5.2: The relative importance of different prey taxa found in the stomach contents of *R. holubi* caught in the four different habitat types for all seasons combined. %*V*= percentage volume, %*F* = percentage frequency of occurrence, %RI = percentage ranking index. The four most important food items per food category (excluding 'unidentified') are shown in bold.

Prey Item	**Mud flats (n=120)**			**Sand flats (n=120)**			**Salt marsh (n=120)**			**Seagrass (n=120)**			**Total (n=480)**		
	% V	% F	% RI	% V	% F	% RI	% V	% F	% RI	% V	% F	% RI	% V	% F	% RI
Annelida															
Polychaeta	0.35	5.83	<0.1	0.70	5.0	<0.1	1.32	4.17	0.11	1.13	3.33	<0.1	1.05	4.58	0.11
Arthropoda															
Anomura	1.44	4.17	0.10	2.66	4.17	0.28	2.30	**5.84**	0.26	**5.29**	6.67	**0.78**	2.88	5.21	0.33
Amphipoda	2.79	**20.00**	**0.91**	<0.1	1.67	<0.1	0.26	5.83	<0.1	1.23	5.86	0.16	1.14	**8.33**	0.21
Brachyura	2.04	6.66	0.24	2.51	1.77	0.16	**5.88**	10.00	**0.57**	**7.82**	**12.5**	**1.13**	**4.57**	7.1	**0.39**
Copepoda	**4.96**	10.00	**0.81**	<0.1	0.83	<0.1	<0.1	<0.1	<0.1	-	-	-	1.27	2.71	<0.1
Mysida	0.86	6.67	<0.1	-	-	-	-	-	-	1.29	2.50	<0.1	0.55	2.29	<0.1
Isopoda	0.15	1.67	<0.1	<0.1	0.83	<0.1	0.81	3.33	<0.1	<0.1	0.83	<0.1	0.28	1.67	<0.1
Insecta	-	-	-	-	-	-	<0.1	0.83	<0.1	-	-	-	<0.1	0.21	<0.1
Palaemonoidea	0.24	1.67	<0.1	0.41	1.61	<0.1	0.57	1.61	<0.1	0.21	1.61	<0.1	0.35	1.67	<0.1
Penaeidae	<0.1	0.83	<0.1	0.11	0.83	<0.1	1.23	2.5	<0.1	0.47	0.83	<0.1	0.43	1.25	<0.1
Mollusca															
Bivalvia	-	-	-	-	-	-	<0.1	0.83	<0.1	-	-	-	<0.1	0.21	<0.1
Gastropoda	**2.80**	**15.0**	0.68	**6.11**	**20.00**	**3.06**	**3.75**	**20.00**	**1.45**	1.42	**15.0**	0.47	**3.51**	**17.50**	**1.37**
Plantae															
Red filamentous algae	**57.88**	**82.5**	**77.57**	**40.1**	**53.3**	**53.4**	**16.0**	**35.0**	**11.0**	**45.3**	**64.2**	**64.3**	**40.2**	**58.8**	**52.32**
Seagrass	**22.10**	**50.0**	**17.94**	**26.9**	**51.7**	**34.82**	**56.6**	**72.5**	**79.4**	**29.35**	**45.0**	**29.33**	**33.5**	**54.8**	**40.88**
Other															
Bryozoa	<0.1	0.83	<0.1	**12.3**	**18.3**	**5.56**	-	-	-	-	-	-	2.92	4.79	0.31
Pisces	-	-	-	1.03	0.83	<0.1	-	-	-	-	-	-	0.24	0.21	<0.1
Unidentified	4.29	75.0	1.74	7.15	15.0	2.68	10.98	33.33	7.08	6.40	24.17	3.44	7.25	24.38	3.9

There were seasonal differences in the general diet of *R. holubi* (tables A5–A8). Although red filamentous algae and seagrass dominated the diet of *R. holubi* in each season, filamentous algae generally had a higher volume, frequency of occurrence and relative importance in spring, summer and winter, while seagrass had a higher volume, frequency of occurrence and relative importance than red filamentous algae in autumn. Polychaeta, brachyura and amphipoda were prominent in the diet of *R. holubi* in each season but in terms of volume and frequency of occurrence constituted a relatively smaller proportion of the diet in winter. Anomura were present in the diet of *R. holubi* in spring and autumn but were largely absent in summer and winter. Mysida occurred in the diet of *R. holubi* in summer only, whilst isopoda and palaemoidea occurred in the diet of *R. holubi* in spring and summer but were absent in autumn and winter.

There were no significant seasonal differences in the diet of *R. holubi* from the mud flats ($p < 0.05$, $R = 0.06$), sand flats ($p < 0.05$, $R = 0.11$), seagrass habitat ($p < 0.05$, $R = 0.02$) or salt marsh beds ($p<0.05$, $R=0.13$). When the seasonal data were grouped by habitat there was no significant difference in the diet of *R. holubi* between habitat types ($p<0.05$ and $R=0.07$).

Diatom assemblage in the seagrass habitat

A total of 55 diatom species were identified from the seagrass leaves (Table 5.3). In spring 2013 the diatom assemblage was dominated by *Navicula* sp. 13 (19.3%), *Grammatophora* sp. 2 (12.9%) and *Pinnularia* sp. 10 (9.8%). In contrast, the summer assemblage was dominated by *Fragilaria* sp. 1 (24.9%), *Pinnularia* sp. 5 (12.8%) and *Grammatophora* sp. 1 (7.2%). In autumn, the assemblage was dominated by *Navicula* sp. 10 (15%), *Nitzchia closterium* (13.1%) and *Pinnularia* sp. 5 (23.7%) and in winter by *Nitzchia* sp. 2 (18.6%), *Pinnularia* sp. 3 (10.2%) and *Navicula* sp. 2 (10%).

Diatom composition in the diet

A total of 45, 27, 23 and 20 species of diatoms were identified in diets of fish from the seagrass, salt marsh, mud flat and sand flat habitats, respectively. *Grammatophora* spp. 1 and 2 were the most abundant diatom species occurring in the diet of fish captured in all habitats. *Nitzshia* sp. 2 was the third most abundant diatom species in the diet of fish caught in the mud flats and seagrass, while *Licmophora* spp. 1 and 2 were the third most abundant diatoms in the stomachs of fish caught in the salt marsh and sand flats, respectively.

ANOSIM results indicated that the composition of diatoms occurring in the foregut of *R. holubi* caught in the sand flats, mud flats, seagrass and salt marsh habitats, and those identified on the leaf blades in the seagrass bed were not significantly different ($R = 0.16$ $P<0.05$).

In spring and summer, *Grammatophora* sp.1 and *Grammatophora* sp. 2 were the most abundant diatoms in the diet of fish caught in each habitat type. These species were, however, considerably less abundant on seagrass leaves; seventeen species in spring and eighteen species in summer identified on the leaves were not present in the diatom assemblage in the stomachs of fish caught (Table 5.3).

In autumn, *Grammatophora* sp. 1 and *Grammatophora* sp. 2 were again dominant in the diet of fish captured in the sand flats, mud flats and salt marsh but were absent in the diets of fish captured in the seagrass habitat and absent from the seagrass leaves. *Licmophora* sp. 2 occurred in high abundance in the stomachs of fish captured in the sand flats and mud flats and *Pinnularia* sp. 1 occurred in high abundance in the mud flats and salt marsh. Both species were absent in the seagrass habitat. Eight species of diatoms identified in the seagrass habitat were not present in the diatom assemblage in the stomach of fish caught. *Pinnularia* sp. 5 was the most abundant diatom in the seagrass habitat; this species was also found in the stomachs of fish captured in the seagrass habitat but was absent in the fish captured in the other habitat types.

In winter, *Nitzchia* sp. 2 was the most abundant diatom species in the stomachs of fish captured in the sand flats, mudflats and seagrass and occurred in a lower abundance in the stomachs of fish from the salt marsh habitats. *Licmophora* sp. 1 and *Licmophora* sp. 3 were abundant in the stomachs of salt marsh fish but were absent in the seagrass habitat. *Grammatophora* sp. 1 and *Grammatophora* sp. 2 were abundant in the stomachs of fish captured in the sand flats and salt marsh but both were absent in seagrass. *Navicula* sp. 4 and *Pinnularia* sp. 7 were highly abundant in the stomachs of fish captured in the mud flats but were absent in the seagrass bed. Eight species of diatoms identified from the seagrass leaves were not present in the diatom assemblage in the stomach of fish caught in each habitat.

Table 5.3: The relative abundance (%) of diatom species observed in the diet of *R. holubi* captured in four habitat types in spring, summer, autumn and winter (2013–2014) and in the *Z .capensis* habitat of the Bushmans Estuary. Values highlighted in bold were species that contributed 10% or more to the diatom assemblage. Values in italics were common to all foreguts and in the seagrass habitat within a season (Diet SF = diet of fish in the sand flats; Diet MF = diet of fish in the mud flats, Diet *S. mar* = diet of fish in salt marsh, Diet *Z. cap.* = diet of fish in seagrass).

Diatom species	**Spring**					**Summer**					**Autumn**					**Winter**					**All Seasons**				
	Diet SF	Diet MF	Diet *S. mar*	Diet *Z. cap*	Habitat *Z. cap*	Diet SF	Diet MF	Diet *S. mar*	Diet *Z. cap*	Habitat *Z. cap*	Diet SF	Diet MF	Diet *S. mar*	Diet *Z. cap*	Habitat *Z. cap*	Diet SF	Diet MF	Diet *S. mar*	Diet *Z. cap*	Habitat *Z. cap*	Diet SF	Diet MF	Diet *S. mar*	Diet *Z. cap*	Habitat *Z. cap*
Amphora sp. 1	-	-	-	-	-	-	-	-	-	-	-	3.0	-	-	-	-	-	-	-	-	-	0.8	-	0.1	-
Amphora sp.2	2.0	-	-	0.4	1.1	-	-	2.5	0.7	2.9	-	-	-	0.4	0.5	-	-	-	-	-	0.5	-	0.6	0.4	1.1
Campylodiscus sp.1	-	-	-	0.1	-	-	-	-	-	0.1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Cocconeis sp. 1	-	-	-	-	0.5	-	-	-	-	0.2	-	-	-	-	0.1	-	-	-	-	-	-	-	-	-	0.2
Cocconeis sp. 2	1.5	-	-	0.1	0.2	-	-	1.0	0.3	0.5	-	1.0	-	0.9	2.1	-	-	-	0.4	1.4	0.4	-	0.3	0.4	1.1
Cocconeis sp.3	-	-	-	-	0.1	-	-	-	-	-	-	-	-	-	0.2	-	-	-	-	-	-	0.3	-	-	0.1
Coscinodiscus sp.1	-	-	-	-	-	-	-	-	-	1.4	-	-	-	0.1	-	-	-	-	-	-	-	-	-	-	0.4
Diatoma vulgaris	-	-	-	-	-	-	-	-	-	0.1	-	-	-	-	-	-	-	-	-	0.3	-	-	-	-	0.1
Diploneis sp. 1	-	-	-	-	0.1	-	-	-	-	0.6	-	-	-	-	--	-	-	-	-	-	-	-	-	-	0.2
Diploneis sp.2	-	-	-	-	3.6	--	2.0	-	-	0.2	3.0	-	1.5	0.4	0.2	-	-	1.6	-	1.8	0.8	-	0.8	0.1	1.5
Diploneis sp.3	-	-	-	-	-	-	-	-	0.4	0.1	-		-			-			-	-	-	0.5		0.1	-
Fragilaria sp. 1	*6.0*	*1.5*	*1.0*	*2.3*	*3.6*	*3.0*	*2.5*	*3.0*	*0.2*	***24.9***	8.0	4.0	-	0.3	0.1	-	-	-	-	-	4.3	-	1	0.7	7.2
Fragilaria sp.2	-	-	-	-	0.2	-	-	-	-		-	-	-	-	0.3	-	-	-	0.7	0.3	-	2	-	0.2	0.2
Fragilaria sp.3	-	-	-	-	-	-	-	-	-	2.0	-	-	-	0.7	-	-	-	-	-	-	-	-	-	0.2	0.5
Gomphonema sp.1	-	-	-	-	-	-	-	0.5	-	0.4	-	-	-	-	0.1	-	-	-	-	-	-	-	0.1	-	0.1
Grammatophora sp.1	***34.0***	***23.5***	***44.5***	***18.9***	***5.3***	***61.5***	***30.5***	***59.0***	***33.2***	***7.2***	**19.0**	**11.5**	**18.5**	-	-	**12.0**	-	**22.5**	-	-	**31**	**16-**	**31**	**13**	3.1
Grammatophora sp.2	***26.0***	***29.0***	***19.2***	***58.6***	***12.9***	***32.5***	***42.5***	***24.0***	***56.3***	***5.1***	**38.5**	**14.0**	**14.5**	-	-	**16.0**	-	**10.0**	-	-	**28**	**21**	**17**	**29**	4.5
Licmophora sp.1	-	-	-	-	0.7	-	-	-	0.1	0.1	-	3.5	-	-	-	7.5	-	**40.5**	-	-	1.9	0.9	10.	0.7	0.2

Diatom species	**Spring**					**Summer**					**Autumn**					**Winter**					**All Seasons**				
	Diet SF	Diet MF	Diet *S. mar*	Diet *Z. cap*	Habitat *Z. cap*	Diet SF	Diet MF	Diet *S. mar*	Diet *Z. cap*	Habitat *Z. cap*	Diet SF	Diet MF	Diet *S. mar*	Diet *Z. cap*	Habitat *Z. cap*	Diet SF	Diet MF	Diet *S. mar*	Diet *Z. cap*	Habitat *Z. cap*	Diet SF	Diet MF	Diet *S. mar*	Diet *Z. cap*	Habitat *Z. cap*
Licmophora sp.2	*6.5*	***12.0***	***15.5***	*0.9*	*1.6*	*1.5*	***12.5***	*2.0*	*1.2*	***2.7***	***19.5***	***15.5***	*1.0*	*0.8*	*1.5*	-	-	-	-	1.8	6.9	10	4.6	-	1.9
Licmophora sp.3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	15.0	-	-	-		3.8	0.5	-
Licmophora sp.4	5.5	7.0	-	-	0.1	1.0	6.5	-	0.8	1.8	1.5	3.0	0.5	1.1	-	-	-	-	-	-	2	4.1	0.1	1.6	0.5
Melasira sp.1	-	-	-	-	0.1	-	0.0	-	-	-	-	-	-	0.1	0.2	-	-	-	-	-	-	-	-	-	0.1
Navicula sp. 1	1.0	1.5	-	2.9	4.6	-	0.5	3.0	0.2	6.9	-	3.0	1.0	-	-	7.0	-	-	3.1	8.9	2	1.3	1	0.1	5.1
Navicula sp.2	-	-		0.4	-	-	-	-	-	-	-	-	-	-	-		-	-	-	10.0				**0.9**	**2.5**
Navicula sp.3	0.5	4.0	-	0.4	0.3	-	-	-	-	0.6	1.5	1.0	9.0		3.2	**15.5**	-	-	3.3	4.2	4.4	1.3	2.3	0.9	2.1
Navicula sp 4	-	-	-		-	-	-	-	-	-	-	-	-	5.8	-		**13.5**	1.0	-	-	-	3.4	0.3	1.5	-
Navicula sp.5	-	-	-		-	-	1.5	-	-	-	-	-	-	-	0.1	-	-	-	-	-	-	0.4	-	-	-
Navicula sp.6	-	-	-	0.1	0.6	-	-	-	-	-	-	-	-	0.1	-	-	-	-	-	0.1	-	-	-	0.1	0.2
Navicula sp.7	-	-	-	1.9	2.0	-	-	-	-	0.1	-	-	-	0.1	5.6	-	-	-	0.2	-	-	-	-	0.6	1.9
Navicula sp.8	-	-	2.0	-	1.1	-	-	0.5	-	0.3	-	-	-	-	3.2	-	1.0	-	-	-	-	0.3	0.6	-	1.2
Navicula sp.9	0.5	-	0.5	-	0.2	-	-	-	-	1.8	-	-	6.0	**12.0**	1.2	-	-	-	-	-	0.1		0.6	3	0.8
Navicula sp.10	-	2.5	-	-	3.3	-	-	-	-	4.9	-	-	-	-	**15.0**	-	-	-	0.3	3.3	-	0.6	1.6	0.1	**6.6**
Navicula sp.11	-	-	-	-	0.4	-	-	-	-	1.6	-	-	6.5	-		-	-	-	-	0.1	-	-	-	-	0.5
Navicula sp.12	-	-	-	-	2.3	-	-	-	-	-	-	-	-	6.4	2.1	-	-	-	-	-	-	-	-	1.6	1.1
Navicula sp13	*6.0*	*6.5*	*0.5*	*6.7*	**19.3**	-	-	-	-	-	2.0	-	-	2.5	6.9	-	-	-	0.2	7.3	2	1.6	1.6	2.4	0.4
Navicula sp.14	-	-	-	-	-	-	0.5	-	-	-	-		-	-		-	-	-	1.9	3.7	-	0.1	-	0.5	0.9
Navicula sp.15	-	-	4.5	-	-	-	-	1.5	-	0.4	-	-	5.5	-	4.7	-	-	1.0	1.5	0.9	-	-	0.1	0.4	1.5
Navicula sp.16	-	-	-	-	2.7	-	-	-	0.2	1.2	-	-	-	**12.5**	0.1	-	1.0	-	-	0.8	-	0.3	--	3.2	1.2
Nitzchia closterium	-	-	-	0.8	8.6	-	-	-	-	5.8	-	-	-	1.8	**13.1**	-	-	-	1.9	2.7	-	-	3.1	1.1	7.6
Nitzchia sp.2	-	-	2.5	-	0.2	-	-	-	-	-	-	-	8.5	-	-	**27.0**	**62.0**	6.1	**68.9**	**18.6**	**0.8**	**15**	-	**17**	4.7
Pinnularia sp. 1	-	-	-	-	0.1	-	-	-	-	-	5.5	**34.5**	**16.0**	2.4	-	-	-	1.5	4.8	6.7	1.4	8.6	-	1.8	1.7
Pinnularia sp. 2	-	-	-	-	0.2	-	-	-	-	1.9	-	4.5	-	1.2	-	-	-	-	-	0.4	-	1.1	4.3	0.3	0.6
Pinnularia sp.3	-	-	-	-	-	-	-	-	-	-	-	-	-		-	-	-	-	0.8	10.2	-	-	4.4	0.2	2.6
Pinnularia sp.4	-	-	-	-	0.1	-	-	-	-	-	-	-	-	**16.8**	-	-	-	-	-	-	-	-	-	4.2	-

Diatom species	**Spring**					**Summer**					**Autumn**					**Winter**					**All Seasons**				
	Diet SF	Diet MF	Diet *S. mar*	Diet *Z. cap*	Habitat *Z. cap*	Diet SF	Diet MF	Diet *S. mar*	Diet *Z. cap*	Habitat *Z. cap*	Diet SF	Diet MF	Diet *S. mar*	Diet *Z. cap*	Habitat *Z. cap*	Diet SF	Diet MF	Diet *S. mar*	Diet *Z. cap*	Habitat *Z. cap*	Diet SF	Diet MF	Diet *S. mar*	Diet *Z. cap*	Habitat *Z. cap*
Pinnularia sp.5	-	-	1.5	-	4.7	-	-	-	-	**12.8**	-	-	-	7.4	**23.7**	2.0	-	-	-	1.6	0.5	-	-	1.9	10.7
Pinnularia sp.6	-	-	-	-	-	-	-	-	-	-	-	-	-		0.7	-	-	-	-	0.6	-	-	-	-	0.3
Pinnularia sp.7	-	-	-	-	-	-	-	-	-	-	-	-	-	0.1	0.2	-	**17.0**	-	-	-	-	4.3	0.4	-	0.1
Pinnularia sp.8	-	-	-	-	-	-	-	-	-	-	-	-	2.5	-	-	-	-	-	-	-	-	1	-	-	-
Pinnularia sp.9	-	3.5	-	-	0.9	-		2.5	-	-	-	-	3.5	**11.8**	2.8	-	0.5	-	3.7	8.2	-	3	-	3.9	3
Pinnularia sp.10	8.0	9.0	7.5	-	9.8	-	1.0	-	0.1	6.1	*0.5*	*0.5*	***5.5***	*0.2*	*4.7*	-	1.5	-	6.8	5.6	2.1	-	0.6	1.8	6.6
Pinnularia sp.11	-	-	-		0.3	-	-	-	-	0.6	0.5	-	-	0.6	0.2	3.0	-	0.5	1.4	-	0.9	-	1.5	0.5	0.3
Pinnularia sp.12	-	-	-	-	6.3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3.3	-	1.6
Surirella sp.1	-	-	-	-	-	-	-	0.5	-	-	-	-	-	7.9	0.2	-	-	-	-	-	-	-	0.1	2	0.1
Surirella sp.2	-	-	-	-	-	-	-	-	6.2	0.1	-	-	-	-	-	-	-	-	-	-	-	-	-	1.6	-
Tabularia sp.1	-	-	-	-	-	-	-	-	0.0	-	-	-	-	-	-	-	-	-	-	-	-	0.6	-	-	-
Unidentified sp. 1	-	-	-	2.4	-	-	-	-	-	3.7	-	-	-	0.1	-	-	2.5	-	-	0.4	-	-	-	0.6	1.0
Unidentified sp. 2	2.5	-	-	-	2.5	0.5	-	-	0.2	0.1	0.5	-	-	-	-	-	-	0.5	-	-	0.9	-	0.1	0.1	0.7
Unidentified sp. 3	-	-	-	-	-	-	-	-	-	1.1	-	-	-	3.7	7.7	-	-	-	-	-	-	-	-	0.9	2.2
Unidentified sp.4	-	-	-	-	-	-	-	-	-	0.5	-	1.0	-	1.6	0.1	10.0	-	-	-	-	2.5	0.3	-	0.4	-
Unidentified sp.5	-	-	-	-	-	-	-	-	-	-	-	0.5	-	0.8	0.1	5.0	-	-	-	-	1.3	0.1	-	0.2	-

In spring, the diatom assemblage of the seagrass habitat was most similar to the diet of fish in the salt marsh habitat and least similar to the mud and sand flat habitats (Fig.5.2).

In summer, the diatom assemblage on the leaf blades in the seagrass habitat was 33.1% similar to the diatoms in the diets of *R. holubi*. The diatoms occurring in the foreguts of *R. holubi* in salt marsh, the sand flats, in the seagrass bed and in the mud flats were between 63.7% and 72.1% similar to each other.

In autumn, the diatom assemblage in the foreguts of *R. holubi* caught in seagrass was most similar (42.9%) to the diatom assemblage occurring on the leaf blades of seagrass 42.91%. The diatoms in the foreguts of *R. holubi* caught in salt marsh, the sand flats and the mud flats were more similar to each other than the diatom assemblage in the foreguts and on the leaf blades of seagrass, with the diatoms in the foreguts from the mud flats and sand flats 60.8% similar to each other.

In winter, the diatom assemblage in the foreguts of *R. holubi* caught in seagrass was 60.2% similar to the diatom assemblage occurring on the leaf blades of seagrass in the same seagrass bed, whilst the diatoms occurring in foreguts of *R. holubi* caught in salt marsh and the sand flats were only 39.7% similar, and the diatoms occurring in the foreguts of *R. holubi* caught in the mud flats were only 32.17% similar to the diatoms in the seagrass bed and in the foreguts of *R. holubi* caught in seagrass.

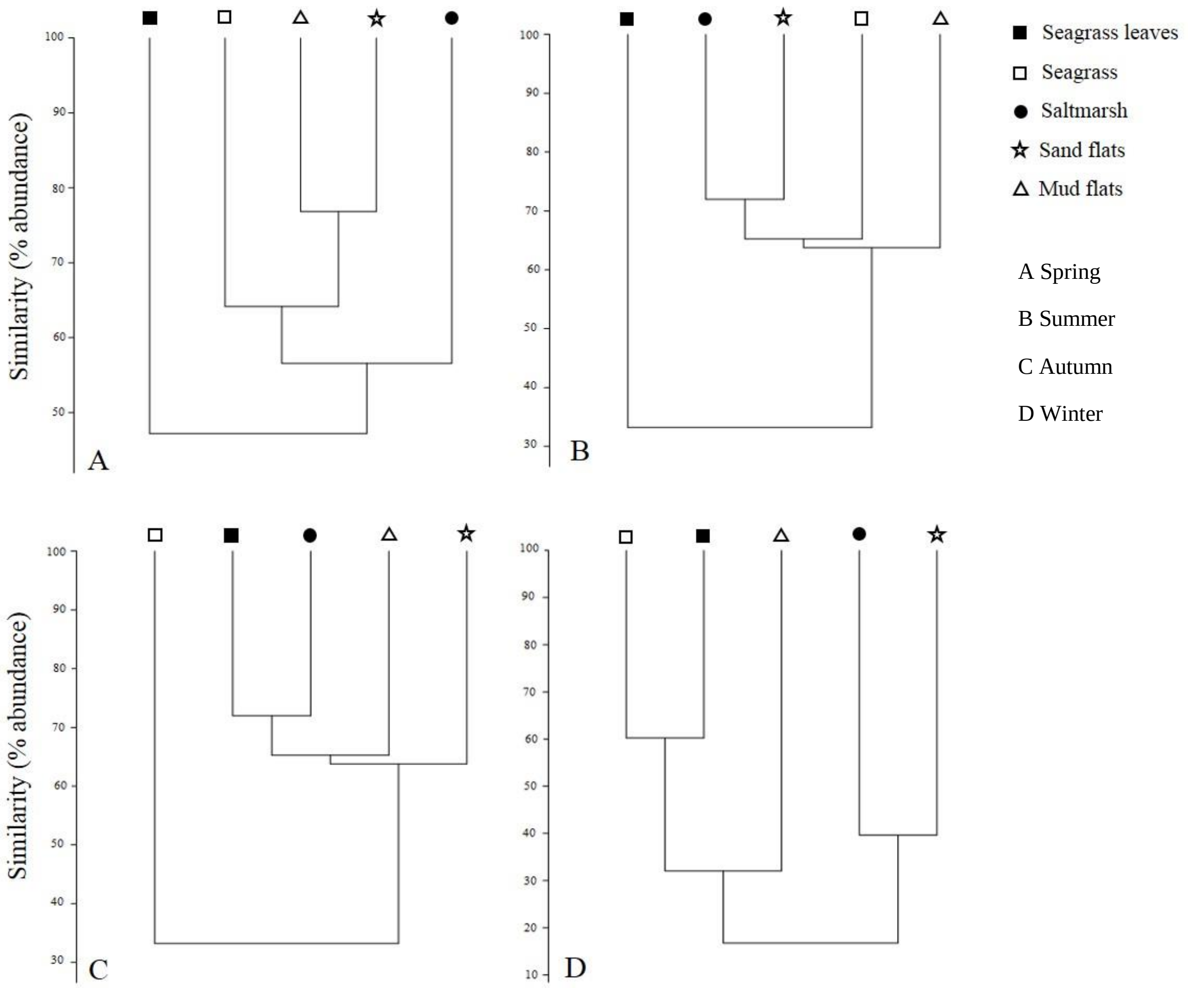

Figure 5.2: Similarity dendogram showing the % similarity of the diatom assemblages found in the foregut of *R. holubi* caught in the mud flats, sand flats, salt marsh beds and seagrass beds and the diatom assemblage occurring on the leaf blades in the seagrass habitat in spring 2013 and summer, autumn and winter 2014

DISCUSSION

Stomach content analysis and dietary diatom composition revealed that *R. holubi* probably feed in all of the main habitats, indicating that *R. holubi* is a generalist and opportunistic feeder that does not specifically rely on any environment for food and may instead rely on suspended plant matter in the water column, as well as on invertebrates occurring in seagrass, salt marsh, sand flat and mud flat habitats. The variation in the invertebrate component of the diet of *R. holubi* caught between habitat types might reflect differences in resource availability and/or resource utilization. However, this conclusion was based on the assumption that the prey items found in the foreguts were preyed upon in the habitat type in which *R. holubi* were captured.

General diet

Plant material (red filamentous algae and seagrass), dominated the diet of *R. holubi* from all habitat types. This is comparable to other studies in Eastern Cape estuaries, where seagrass and filamentous algae comprised up to 90% of the diet of *R. holubi* (Blaber 1973, de Wet and Marais 1990, Whitfield 1998). Red filamentous algae such as *Polysiphonia* sp. has been found to occur within the littoral areas in the Swartvlei Estuary, and in this estuary a strong positive relationship existed between the presence of episammic filamentous algal mats (*Chaetomorpha* sp. and *Polysiphonia* sp.) and the abundance of *R. holubi* (Whitfield 1984). Although no such algal mats were observed in the Bushmans Estuary during this study, nor were any observed in a previous review of the aquatic macrophytes in the estuary (Jafta 2010), Blaber (1974) found that red algae, which was washed into the Kowie Estuary from the sea, was consumed by *R. holubi* and he suggested that this was an abundant source of marine diatoms.

Although the volumetric contribution of invertebrate prey items was less than 5% of the diet, the high overall frequency of occurrence of invertebrate prey items (45.62%) and broad range of invertebrate prey items underlines the contribution and importance of invertebrates in the diet of *R. holubi* in the Bushmans Estuary. Other studies have also found a broad range of invertebrate prey groups in the diet of *R. holubi*, with large variation in the contribution of invertebrates to the diet between estuaries and seasons (e.g. Blaber 1973, Whitfield 1984, Whitfield 1988, Schlacher and Wooldridge 1996). These findings support the conclusion that *R. holubi* is a generalist invertebrate feeder behaviour that feeds on the invertebrates that are most abundant in the estuary.

Sparids do not have the digestive enzyme cellulase and hence cannot digest plant matter with cell walls (Blaber 1973). Only epiphytic diatoms and other algae with fragile cell walls can be digested and assimilated, while the macrophyte component passes through the gut undigested (Blaber 1973, de Wet and Marais 1990). Furthermore, 73% of diatoms consist of indigestible silaceous frustules (Blaber 1973). Hence, as *R. holubi* is not effective at digesting plant matter, the invertebrate contribution to the diet of *R. holubi*, although low in terms of volume, is probably critical from a nutritional perspective. Sparids, including species such as European seabass (*Dicentrarchus labrax)*, gilthead seabream (*Sparus aurata)* and common dentex (*Dentex dentex*) require a diet constituting between 46% and 55% protein. Diatoms are rich in lipids, including glycolipids, glycerides and poly-unsaturated fatty acids (Kates and Volcani 1966, Dunstan et al. 1996). Lipid content of between 9% and 17% in the diet has been found to enhance protein retention and increase weight gain in sparids (Pavlidis and Mylonas 2011).

Pavlidis and Mylonas (2011) also assert that it is the combination of protein and lipids that is crucial for sparid growth. Hence it is probably the combination of protein from invertebrates and lipids from diatoms that is critical for the growth of juvenile *R. holubi* in nursery habitats.

There were broad similarities in the diet of *R. holubi* caught in the four habitat types of the Bushmans Estuary. In terms of volume, frequency of occurrence and relative importance, plant matter dominated the diet of *R. holubi* captured in each habitat type. Red filamentous algae comprised the dominant food item in all habitats. This was unsurprising since this marine seaweed drifts freely in the water column and is probably equally available to fish throughout the lower and middle reaches of the estuary. Seagrass was a frequent component in the diet of *R. holubi*. Hence seagrass beds may be an important feeding habitat for all *R. holubi*, with fish frequently moving to and from this habitat for feeding. In autumn and winter the diatom assemblage in the stomachs of *R. holubi* partially reflected the diatom assemblage occurring on the seagrass leaf blades in the seagrass habitat. Alternatively, *R. holubi* may feed opportunistically on seagrass leaves that have become detached and suspended in the water column while they are feeding in other estuarine habitats.

Critically, there were important differences in the assemblage of food items in the stomachs of *R. holubi* caught in the different habitats. The dissimilarities in the food items identified in the stomachs of fish captured in each habitat type may indicate differences in resource availability and/or resource utilization in each habitat type. If *R. holubi* fed only on invertebrate food items within seagrass, the invertebrate food items found in the stomachs of fish caught in the various habitat types would be expected to be the same. To some extent this was true: invertebrate species such as *Hymenosoma orbiculare*, *Palaemon pacificus*, *Melita zeylanica* and *Assiminea* sp. were identified in the stomachs of *R. holubi* caught in each habitat type and are known to inhabit submerged aquatic macrophyte beds, such as seagrass (Whitfield 1989b, Branch et al. 2010). However, other invertebrate prey species, such as polychaeta, copepoda, mysida, panaeidae and bryozoa, were often either found to occur in the diets of fish caught in other habitats and not in the stomachs of fish caught in seagrass, or alternatively were found to occur in the diets of fish caught within seagrass and not in the diets of fish caught in the other habitats.

In the Swartvlei Estuary distinct invertebrate assemblages have been found to occur in seagrass and sand flat habitat types (Whitfield 1989b) and this may also be the case in the Bushmans Estuary. *Callianassa krausii* and *Upogebia africana,* which were identified in the diet of *R. holubi,* inhabit sand flats and mud flats respectively (Branch et al. 2010, Whitfield 1989b), indicating that *R. holubi* may be feeding on anomura in these habitat types. *Rhabdosargus holubi* has been found to move extensively between areas in the Grants Valley Estuary in the south-east Cape coast of South Africa (Lukey et al. 2006). Hence, *R. holubi* is probably a generalist opportunistic feeder that does not rely on any specific environment for food. It feeds actively on invertebrates salt marsh, seagrass and in muddy and sandy substrata, but seems to rely on plant matter that is suspended in the water column.

Seasonal trends in the diet of *R. holubi* indicate that feeding on a broad range of invertebrates occurred more frequently and at higher volumes in the summer months. The general dietary breadth dropped drastically in winter and was dominated by red filamentous algae. This may be a result of a reduction in the availability of prey items or a result of slower movement in colder temperatures resulting in a reduced ability to capture prey.

Diatom composition analysis

Diatom assemblages are indicators of and are sensitive to environmental parameters such as salinity and nutrient availability within estuaries (Stoermer and Smol 1999, Frankovich et al. 2006). As environmental parameters vary spatially along the length of estuaries (Lancelot and Muylaert 2011), it can be assumed that the diatom composition on the leaf blades of seagrass varies between locations according to differences in environmental conditions in situ. However, this is assuming that the diatom composition in the foreguts reflects the diatom composition found on the leaves of seagrass near each habitat type and that *R. holubi* fed on seagrass near each of the habitats types selected along the length of the estuary. The diatom composition occurring in the stomach of fish captured in each habitat type were of varying degrees of similarity to the diatoms in the sampled seagrass habitat. In spring and summer, there was a large degree of dissimilarity between the diatoms in the stomach of fish captured in all habitat types and the diatoms on the leaf blades within the sampled seagrass habitat. This suggests that *R. holubi* in seagrass may be feeding on the diatom assemblage on seagrass but may also be feeding elsewhere, whilst *R. holubi* occurring in non-seagrass habitats are not feeding in seagrass. In autumn and winter, the diatom composition occurring in the stomach of fish caught in seagrass was more similar to that found in the seagrass habitat, indicating that, in these seasons, *R. holubi* may be feeding in the seagrass habitat more than during spring and summer. However, the diatoms in gut contents of fish caught in the other habitats were dissimilar to the diatoms occurring in the seagrass habitat, indicating that they may be feeding on diatoms in other areas all year round.

Diatoms that were abundant in the seagrass habitat were often either present in low abundance or absent in the stomach of fish caught outside of seagrass. Although there may be a degree of similarity between the diatom composition from the leaves of *Z. capensis* with those found in the stomach of *R. holubi* in each of the habitats, there is little evidence to suggest that seagrass is the primary feeding habitat for this species; instead, it indicates that *R. holubi* is a generalist, opportunistic feeder that feeds within seagrass and other habitats, and is also dependent on plant matter in the water column.

Grammatophora spp. 1 and 2 were the dominant diatoms in the diets of *R. holubi* and were found on the seagrass leaves, but not in great abundance (>5% in all seasons). *Grammatophora* has been described as an epiphytic marine diatom genus (Van Landingham 1971) most frequently found on the surface of filamentous red algae (Rhodophyta) (Sato et al. 2010). As diatoms occur on filamentous algae and *Grammatophora* is highly associated with Rhodophyta (Sato et al. 2010), it is highly likely that a large proportion of *Grammatophora* sp. present in the diets of *R. holubi* were epiphytic to the red filamentous algae ingested by *R. holubi* This suggests that the difference in the diatom assemblage between that found in seagrass and that found in the stomachs of *R. holubi* caught in each habitat type may be due to the diatom assemblage associated with the red filamentous algae ingested by *R. holubi* in each habitat type throughout the year. *Grammatophora* is a marine cosmopolitan planktonic genus with four ribbon-like chromatophores per cell. The lobes of the chromatophores fill the spaces between the septae. This genus is usually associated with the littoral zone of subtropical and temperate seas and may be washed into estuaries from the ocean (Sato et al. 2010). This concurs with the findings of Blaber (1974).

The use of diatoms as an indicator of feeding habitat

The identification of diatoms in the stomachs of *R. holubi* proved to be a useful tool for determining the feeding habitats of *R. holubi*. Diatom assemblage analysis revealed that the source of the diatoms consumed by *R. holubi* occurring within each habitat type is likely to be predominantly from red filamentous algae and from seagrass. This discovery was only possible through identifying the diatoms occurring within the stomach of *R. holubi* and hence illustrates the value of using diatoms as an indicator of feeding habitat

Conclusion

Chapter Three indicated that *R. holubi* abundance was significantly higher in seagrass than salt marsh and sand flat habitats, and slow meandering behaviour exhibited by *R. holubi* in seagrass indicated a higher degree of habitat use than in the salt marsh and sand flat habitat types. This chapter has provided an indication of the importance of different habitats for the feeding of juvenile *R. holubi*. Ultimately, *R. holubi* appears to feed in all of the habitats in which it is found. It also makes extensive use of food sources of a marine origin. The dietary diatom species analysis appeared to be an appropriate method for understanding the resource utilization patterns.

CHAPTER SIX

GENERAL DISCUSSION

Importance of estuarine nursery areas

Estuarine nursery habitats, such as seagrasses, salt marshes and mangroves, provide not only energy rich food resources that contribute towards high specific growth rates (Dahlgren et al. 2006) but also habitat structural complexity resulting in increased survival of estuarine-dependent fish and invertebrate species through protection from predators (Jenkins and Wheatley 1998, Bloomfield and Gillanders 2005, Schaffler et al. 2013). These factors together enhance recruitment of juvenile fish and invertebrates to adult populations (Beck et al. 2001). Habitats that support high densities of estuarine-dependent fish species are critical for maintaining nursery function in estuaries (Elliot and Hemingway 2002, Able 2005). The nursery role hypothesis, as defined by Beck et al. (2001), has provided an approach to assess the relative importance of nursery habitats for juvenile fish and invertebrates. Of the four factors suggested by Beck et al. (2001): density, growth, survival of juveniles and movement to adult habitats, most studies have focused on density because of the difficulty involved in obtaining measurements of the other factors. In this study, a different multi-method approach was used as an alternative measurement of the nursery role of habitats in the Bushmans Estuary.

The value of estuarine habitats in the Bushmans Estuary as nursery areas for R. holubi

Although previous research has suggested that *R. holubi* is a vegetation-associated species (e.g., Sheppard et al. 2012), there is little empirical evidence indicating which habitat types are used by *R. holubi* for feeding and/or for protection from predators. The multi-method approach used in this study, which included an assessment of habitat complexity, fish abundance and behaviour, diet and diatoms as indicators of feeding habitat, provided insight into the possible roles of different estuarine habitats as nursery areas for juvenile *R. holubi*.

The significantly higher relative abundance of *R. holubi* in seagrass than salt marsh suggests that seagrass is the nursery habitat responsible for a higher number of *R. holubi* sub-adults joining adult populations rather than salt marsh and sand flats, because relative abundance is related to density and density is indicative of emigration, recruitment and mortality (Minello 1999). The high incidence of slow meandering fish behaviour in seagrass indicated a higher degree of habitat use in seagrass than in salt marsh and sand flats, suggesting that *R. holubi* spend more time within seagrass habitats than other habitats. The stem density and multiple dimensionless indices (ISI, C_t/A_t and fractals) used in Chapter Two consistently indicated that the degree of complexity is higher in seagrass than in salt marsh and sand flats, regardless of scale. Habitat types that are of higher complexity provide greater refuge value (Hovel and Lipcius 2001, Jackson et al. 2006). Hence, it can be assumed that seagrass provides better protection from predators than salt marsh and sand flats in the Bushmans Estuary and that greater protection from predators in seagrass supports greater survival. Structurally complex habitats also allow for more time for feeding, and thus species can be expected to exhibit greater growth rates in structured habitats than in unstructured habitat. Structurally complex habitats also provide more substrate for food to grow, again influencing growth rates (Heck et al. 2003.

It must be noted that salt marsh also offers habitat complexity for *R. holubi*, but not as much as seagrass. However, as abundances in salt marsh were comparable to that of sand flats, there is little evidence to suggest that *R. holubi* frequently use salt marsh for protection from predators and hence salt marsh may not be an important nursery habitat for *R. holubi* in the Bushmans Estuary. The dominant behaviour of *R. holubi* in salt marsh was rapid swimming, indicating a low degree of habitat use. Furthermore, the salt marsh habitat in the Bushmans Estuary fell within the emergent tidal zone and was only inundated mid-way through high tide (approximately 0–0.4 m) and spring high tides (approximately 0.4–0.85 m). During neap, low and spring low tides, salt marsh is not available for use for *R. holubi*, whilst seagrass and sand flats are available during all tidal cycles. This indicates that *R. holubi* cannot use salt marsh for protection from predators or for foraging for long periods of time during tidal cycles and will need to use other habitats for foraging and protection from predators. This, combined with the low abundance and behaviour of *R. holubi* in salt marsh, suggests that salt marsh is not a major nursery habitat for *R. holubi* in the Bushmans Estuary. Paterson (1998) noted that *R. holubi* do not inhabit the vegetated portion of salt marsh systems in the Kariega Estuary and despite the complexity provided by *S. maritima*, it may be unlikely that *R. holubi* frequently utilize the vegetated component of salt marsh habitats in other estuaries in the Eastern Cape.

The assessment of diet for *R. holubi* revealed that *R. holubi* is a generalist and opportunistic feeder that does not rely on any specific environment for food and probably feeds on invertebrates and diatoms and algae found on *Z. capensis* floating in the water column and red filamentous algae that is washed into estuarine habitats from the marine environment. As seagrass was not found to be a more important feeding habitat than salt marsh, sand flats and mud flats, it is possible that the high abundance of *R. holubi* in seagrass indicates that *R. holubi* uses seagrass primarily for the complexity it provides.

Estuarine habitats that are not nursery habitats may also provide some resources for juvenile fish species (Beck et al. 2001). The salt marsh, sand flat and mud flat habitats were observed to be dominant habitats that extend over large areas of the Bushmans Estuary and may indirectly provide energy sources for *R. holubi* through suspended filamentous algae and seagrass leaves, as well as invertebrates such as *Callianassa krausii*. Hence the mud flats, sand flats and salt marsh habitats may function as important juvenile habitats that provide a food resource for *R. holubi* but don't necessarily function as nursery areas per se. Furthermore, there is also some evidence to suggest that *S. maritima* salt marsh may be a direct source of carbon for *R. holubi*, although this assertion is contentious. Paterson (1998) suggested that *R. holubi* uses an isotopically enriched source of carbon in the Kariega Estuary that originates from *S. maritima* salt marsh and *Z. capensis* seagrass beds and that salt marsh derived detritus may be utilized by *R. holubi* in the lower reaches of inter-tidal salt marsh creeks. Furthermore, Paterson (1998) noted that *R. holubi* formed a prominent component of the fish assemblage found adjacent to *S. maritima* and *Sarcocornia perennis* salt marsh creeks in the Kariega Estuary. Salt marsh may make some contribution to juvenile *R. holubi* through the availability of resources and may make some contribution towards growth rates and hence recruitment of sub-adults to adult populations. However, further research is needed to understand the extent to which carbon is derived from each habitat before any confident conclusions can be made. It is also important to note that the nursery value of a habitat type occurring in multiple areas may vary spatially. For example the availability of food resources may differ between macrophyte beds of the same species due to differences in structure and functioning and this may influence growth and survival rates as well as recruitment to adult populations (Beck et al. 2001). Factors such as area, edge effects, degree of fragmentation and total patch heterogeneity affect ecosystem dynamics including productivity and species diversity, and these factors are known to vary between seagrass beds (Smith et al. 2012). Hence, the value of an estuarine habitat type may vary depending on the resources a habitat type may provide in areas within an estuary. Furthermore, the proximity of different habitat types to each other may influence the exchange of carbon and nutrients between habitats and therefore affect the nursery value of adjacent habitats as energy sources for fish (Beck et al. 2001).

Current and future impacts on seagrasses

Due to the sensitivity of seagrasses to environmental stressors and direct and indirect anthropogenic impacts in coastal areas, seagrass ecosystems are globally in rapid decline (Duarte 2002, Orth et al. 2006). Sediment and nutrient run-off, invasive species, salinity changes, disease, commercial fishing practices resulting in food web changes, aquaculture, overgrazing and algal blooms are direct impacts that have contributed to global seagrass losses and these factors are predicted to continue to bring further large scale changes to seagrass ecosystems (Duarte 2002, Orth et al. 2006, Hughes et al. 2009). Furthermore, human activity has also been found to cause the fragmentation of seagrass habitats to the point that ecosystem functioning within seagrasses in compromised (Montefalcone et al. 2010). Global climate change is also impacting on seagrasses. The removal of seagrasses through uprooting is predicted to increase with climate change due to erosion associated with sea-level rise and increased wave action associated with storm surges and cyclones (Duarte 2002). Seagrass distribution will also shift in response to changes in water depth and seawater intrusion into estuaries as a result of sea-level rise (Short and Neckles 1999). Climate change is also predicted to affect seagrass productivity and distribution (Short and Neckles 1999). Increased aquatic CO_2 as a result of increased CO_2 emissions may result in higher reproductive output, below-ground biomass and vegetative proliferation of new stems in species such as *Z. marina*, with primary production increasing in localities that are CO_2 limited (Palacios and Zimmerman 2007). The distribution of seagrasses will shift as a result of increased temperature stress and changes in the patterns of sexual reproduction, although this will vary between species (Palacios and Zimmerman 2007). Increased sea temperatures will increase respiration, growth and flowering and increased associated microbial metabolism (Duarte 2002).

Novelty of study

Little or no research on habitat complexity has been conducted in southern African estuaries. This study is the first to use a multi-method approach to assess the nursery value of multiple habitats in a southern African estuary. This study encountered some of the challenges involved in both assessing complexity of multiple habitats and measuring the abundance of fish in vegetated habitats. Kovalenko et al (2012) recommended that habitat complexity studies should incorporate multiple complexity indices in order to provide a thorough analysis of habitat complexity and this study adopted this approach for quantifying the habitat complexity of dominant vegetated habitat types in the Bushmans Estuary. The dimensionless multiple complexity indices made comparisons of complexity between vegetated habitats possible.

Above ground biomass and canopy height have been recognized as inaccurate indicators of habitat complexity as they do not indicate differences in shape of different plant species and hence the true nature of complexity. The higher biomass in salt marsh than seagrass in the Bushmans Estuary suggests that the relative abundance of *R. holubi* should have been higher in salt marsh than seagrass, whilst the relatively similar canopy heights of salt marsh and seagrass indicated that *R. holubi* abundance should be similar in each habitat. However, the fact that relative abundance was significantly higher in seagrass than in salt marsh indicates that stem density and the dimensionless indices may be better tools for assessing habitat complexity and that biomass and canopy height may be unreliable indicators of feeding habitat.

Few studies have quantified both the relative abundance and behaviour of species in multiple habitats in estuaries. Using underwater camera footage allowed for an assessment of the nursery role of multiple estuarine habitat types. Furthermore, few studies have assessed the general diet of individuals caught in various habitats and used diatoms as an indicator of feeding habitat as a method for understanding the feeding role of multiple estuarine habitat types. Assessing abundance and behaviour in combination with the role of feeding habitats in the diet of fish provided an important step towards uncovering the role of nursery habitats for *R. holubi.*

Recommendations and future approaches for nursery role studies

A pressing issue in aquatic science amongst scientists, conservationists and managers is how to prioritize the conservation of habitats in estuaries (Krauss and Secor 2005). Studies that test the nursery role of different habitats in estuaries can provide insight into the relative nursery value of multiple habitat types in estuaries and the findings of these studies can inform ecosystem management and conservation plans (Beck et al. 2001). The relationship between threatened seagrasses and their dependent communities underlines the need for ecosystem based management approaches for effective conservation (Hughes et al. 2009). However, few studies have assessed the nursery role of multiple vegetated habitats as nursery areas for estuarine-dependent marine fish species. Beck et al. (2001) suggests that it is not enough to use single factors in nursery role studies as multifactor experiments provide better insight into factor interactions. However, the majority of studies assessing the nursery value of multiple vegetated habitats have used density and survival as the only indicator of nursery value (Table 6.1).

Table 6.1: Studies assessing the nursery value of two or more vegetated habitat types for juvenile fish using fish density/abundance, growth and survival.

Nursery assessment factor	**Habitat types**	**Location**	**Reference**
Density	Seagrass, mangrove, salt marsh, unvegetated	Barker-Inlet Port Estuary, South Australia	Bloomfield and Gillanders 2005
Density	Salt marsh, seagrass and unvegetated	Aransas Bay and San Antonio Bay, USA	Rozas and Minello 1997
Density	Seagrass, salt marsh edge, non-vegetated	Galveston Bay, Taxas, USA	Stunz et al. 2002
Density	Submerged aquatic vegetation, salt marsh, unvegetated bottom	Atchafalaya River Delta, Louisiana, USA	Castellanos and Rozas 2001
Density	Seagrass, salt marsh creeks, macroalgae,	New Jersey estuaries, USA	Sogard and Able 1991
Density	Mangrove, seagrass, coral reef	Lee Stocking Island, Great Exuma, Bahamas, Mahahual, Mexico, and Turneffe Atoll, Belize	Chittaro et al. 2005
Growth	Salt marsh, seagrass, open water and macro-algae	Navesink River, New Jersey, USA	Phelan 2000
Growth	Seagrass and salt marsh edge	North River, North Carolina, USA	Irlandi and Crawford 1997
Survival	Seagrass, salt marsh, open water	Mobile Bay, Alabama USA	Heck et al. 1994
Survival	Seagrass, salt marsh, open water	Christmas Bay, Texas, USA	Minello 1993
Survival	Sub-tidal salt marsh creek, seagrass	Chesapeake Bay, USA	Ryer et al. 1997

Although some research has been conducted on the functioning of shallow water habitats as refugia for juvenile fishes, e.g., Paterson and Whitfield (2000), little research in southern African estuaries has focused on assessing the nursery role of multiple habitats in an estuary for estuarine-dependent fish species. The approach used in this study for assessing nursery areas for an estuarine-dependent marine fish species provided a useful alternative to that suggested by Beck et al. (2001). Studies quantifying growth, survival and recruitment of sub-adults to adult populations are rare, mainly because of the difficulty involved in obtaining all these measurements. Future studies should concentrate on the nursery value of multiple habitat types within other estuaries in South Africa for *R. holubi,* as well as for other estuarine-dependent fish species. For example, little is known about the nursery role of other vegetated habitat types, such as those dominated by other species of submerged aquatic macrophytes (i.e., *Potamogeton pectinatus* and *Ruppia cirrhosa*) along the eastern and southern Cape coast of South Africa as well as mangrove forests present along the east coast of South Africa northwards of the Nahoon Estuary. Future studies should also aim to address the significance of the variation of multiple plant species and the combination of different vegetation types as nursery areas for fish in estuaries in South Africa.

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APPENDIX

Table A1: A comparison of recorded above-ground biomass of *Zostera capensis*, *Zostera marina*, *Spartina maritima* and *Spartina alterniflora*

Species name	**Above-ground biomass (g.m^{-2})**	**Locality**	**Reference**
Z. capensis	**Spring: 42.3±3.5** **Summer: 65.5±5.3** **Autumn: 38.9±5.18** **Winter: 34.6±3.33**	**Bushmans Estuary**	**Present study**
	Summer:55±21 Winter:105±44	Kromme Estuary	Hanekom and Baird 1984
	217.7	Langebaan Lagoon	Christie 1981
	Summer: 15.7±4.5 Winter: 25.7±8.0	Inhaca Island, Mozambique	de Boer 2000
	7.9-51.3	Inhaca Island, Mozambique	Paula et al. 2001
	Summer: 3-35 Winter: 2-18	Swartkops Estuary	Talbot and Bate 1987
Z. marina	150 - 900	Yealm Estuary, Devon, UK	Attrill et al. 2000
	50 -185	North Carolina, USA	Penhale 1977
S. maritima	Spring: 62.6±16.8 Summer:116.6±1.9 Autumn: 43.38±17.2 Winter: 62.35±13.6	Bushmans Estuary	Present study
S. alterniflora	593±105	Iberian Peninsula, Spain	Castillo et al. 2008
	November: 51±21 March: 116±36	Parangua Bay, Brazil	da Cunha Lana et al. 1991

Table A2: A comparison of recorded stem densities of *Z. capensis*, *Z. marina*, *S. maritima* and *S. alterniflora*

Species name	Stem density (no.m^2)	Locality	Reference
Z. capensis	**761.1±40.08**	**Bushmans Estuary**	**Present study**
	Summer: 2540±427 Winter:2992±517	Inhaca Island, Mozambique	de Boer 2000
Z. marina	40 -160	Yealm Estuary, Devon, UK.	Attrill et al. 2000
	77.2- 385.6 and 66.0 - 516.3	Buzzards Bay, USA and Chesapeake Bay, USA	Wyda et al. 2002
S. maritima	**141.61±25.31**	**Bushmans Estuary**	**Present study**
	500-1800	R´ıa de Betanzos, (Northwest Spain)	Sanchez et al. 2009

Table A3: A comparison of recorded stem length of *Z. capensis*, *Z. marina*, *S. maritima* and *S. alterniflora*

Species name	Stem length (cm)	Locality	Reference
Z. capensis	33±11.5	Bushmans Estuary	Present study
	Summer: 50.4±23.8 Winter: 4.7±24.5	Inhaca Island, Mozambique	de Boer 2000
Z. marina	50-260	Yealm Estuary, Devon, UK	Attrill et al. 2000
S. maritima	40.4±8.3	Bushmans Estuary	Present study
S. alterniflora	43± 10 - 104±9	Parangua Bay, Brazil	da Cunha Lana et al. 1991

Table A4: The ISI results of various plant species in a range of habitat complexity studies

Reference	**ISI value**	**Species name**	**Type**
Dibble et al. 1997	1.5	*Myriophyllum spicatum*	Submergent
Dibble et al. 1997	3.5	*Potamogeton nodosus*	Submergent
Dibble et al. 1997	8.2	*Zosterella dubia*	Submergent
Dibble et al. 1997	12.4	*Egeria densa*	Submergent
Warfe et al. 2008	42.0	*Potamogeton pectinatus*	Submergent
Warfe et al. 2008	0.5	*Eleocharis sphacelata,*	Emergent
Warfe et al. 2008	0.47	*Triglochin procera*	Emergent
Dibble and Thomaz 2006	18.27	*Myriophyllum variifolium*	Submergent
Dibble and Thomaz 2006	3.0	*Nymphaea amazonum*	Floating
Dibble and Thomaz 2006	58.4	*Utricularia foliosa*	Submergent
Dibble and Thomaz 2006	5.7	*Eichhornia auzurea*	Submergent

Table A5: The fractal results of various plant species in two habitat complexity studies

Reference	**Fractal dimension (*D*)**	**Species name**	**Type**
Dibble and Thomaz 2009	25cm^2 magnification= 1.2	*Nymphaea amazonum*	Floating
	25cm^2 magnification= 1.6	*Egeria najas*	Submergent
	25cm^2 magnification=1.7	*Najas conferta*	Submergent
	25cm^2 magnification=1.3	*Cabomba furcata*	Submergent
	25cm^2 magnification= 1.65	*Eichornia azurea*	Submergent
McAbendroth et al. 2005	*D* low magnification = 1.58 *D* high magnification=1.56	*Myriophyllum alerniflorum*	Submergent
	D low magnification = 1.54 *D* high magnification = 1.89	*Potamogeton polygonifolius*	Emergent
	D low magnification = 1.28 *D* high magnification = 1.50	*Juncus bulbosus*	Emergent
	D low magnification = 1.30 *D* high magnification = 1.71	*Juncus articulatus*	Emergent
	D low magnification = 1.46 *D* high magnification = 1.75	*Ranunculus flammula*	Emergent

Table A6: The relative importance of different prey taxa found in the stomach contents of *R. holubi* caught in the four different habitat types in spring. The four most important prey items per prey category (excluding 'unidentified') are shown in bold. %V= percentage volume, %F = percentage frequency of occurrence, %RI = percentage ranking index

Prey Item	Mud flats (n=120)			Sand flats (n=120)			Salt marsh (n=120)			Seagrass (n=120)		
	%V	%F	%RI	%V	%F	%RI	%V	%F	%RI	%V	%F	%RI
Annelida												
Polychaeta	0.75	6.66	0.08	-	-	-	4.28	3.33	0.29	0.37	36.67	0.23
Arthropoda												
Anomura	0.67	10.00	1.10	1.75	3.3	0.12	6.24	**16.67**	2.12	**5.38**	6.67	**0.60**
Amphipoda	**4.09**	**26.66**	**1.66**	0.02	3.33	0.01	0.14	6.67	0.02	-	-	-
Brachyura	**5.19**	10.00	0.79	-	-	-	**4.75**	6.5	**0.65**	**15.82**	**26.67**	**7.08**
Copepoda	-	-	-	-	-	-	-	-	-	-	-	-
Mysida	1.63	10.00	0.25	-	-	-	-	-	-	-	-	-
Isopoda	-	-	-	0.33	3.33	0.02	0.44	3.33	0.03	-	-	-
Insecta	-	-	-	-	-	-	-	-	-	-	-	-
Palaemonoidea	0.25	3.33	0.01	-	-	-	-	-	-	0.84	6.67	0.09
Penaeidae	0.11	3.33	0.01	-	-	-	5.17	**10.00**	1.05	-	-	-
Mollusca												
Bivalvia	-	-	-	-	-	-	-	-	-	-	-	-
Gastropoda	3.3	**33.33**	1.67	**3.3**	**23.33**	**1.52**	**6.02**	**16.67**	**2.04**	3.35	**30.00**	1.69
Plantae												
Red filamentous algae	**64.61**	**86.66**	**85.01**	**39.78**	**60.00**	**47.11**	-	-	-	**47.88**	**83.33**	**66.98**
Seagrass	**11.94**	**36.67**	**6.65**	**36.14**	**60.00**	**42.79**	**56.27**	**70.00**	**80.21**	**23.23**	**56.67**	**22.10**
Other												
Bryozoa	-	-	-	**11.36**	**33.33**	**7.47**	-	-	-	-	-	-
Pisces	-	-	-	-	-	-	-	-	-	-	-	-
Unidentified	7.45	33.33	3.77	7.32	6.67	0.96	16.69	40.00		3.14	23.33	1.23

Table A7: The relative importance of different prey taxa found in the stomach contents of *R.holubi* caught in the four different habitat types in summer. The four most important prey items per prey category (excluding 'unidentified') are shown in bold. %*V*= percentage volume, %*F* = percentage frequency of occurrence, %RI = percentage ranking index

Prey Item	Mud flats (n=120)			Sand flats (n=120)			Salt marsh (n=120)			*Seagrass* (n=120)		
	%V	%F	%RI	%V	%F	%RI	%V	%F	%RI	%V	%F	%RI
Annelida												
Polychaeta	0.64	20.00	0.19	**1.12**	**10.00**	0.2	0.47	6.66	0.06	**4.14**	**10.00**	**0.79**
Arthropoda												
Anomura	-	-	-	-	-	-	-	-	-	0.87	3.33	0.06
Amphipoda	2.50	**16.67**	**0.60**	0.08	3.33	0.01	0.78	**13.33**	0.20	0.29	**6.67**	0.04
Brachyura	**2.74**	10.00	0.39	0.42	3.33	0.03	**0.32**	3.33	0.02	**3.71**	3.33	0.24
Copepoda	-	-	-	-	-	-	**0.05**	3.33	0.01	-	-	-
Mysida	0.78	10.00	0.11	-	-	-	0.05	3.33	0.01	3.89	6.67	**0.50**
Isopoda	0.62	6.67	0.06	-	-	-	**3.80**	10.00	**0.59**	0.33	3.33	0.02
Insecta	-	-	-	-	-	-	-	-	-	-	-	-
Palaemo-noidea	0.73	3.33	0.04	**1.11**	3.33	0.07	-	-	-	-	-	-
Penaeidae	-	-	-	-	-	-	-	-	-	-	-	-
Mollusca	-	-	-	-	-	-	-	-	-	-	-	-
Bivalvia	-	-	-	-	-	-	-	-	-	-	-	-
Gastropoda	**5.90**	**20.00**	**1.70**	0.79	**6.66**	**0.01**	**5.26**	**23.33**	**2.34**	0.13	**10.00**	0.03
Plantae	-	-	-	-	-	-	-	-	-	-	-	-
Red filamentous algae	**67.29**	**86.67**	**83.98**	**67.37**	**66.67**	**81.22**	**14.29**	**26.66**	**7.28**	**39.33**	**56.67**	**42.69**
Seagrass	**9.82**	**53.33**	**7.54**	**16.71**	**53.33**	**16.12**	**58.94**	**70.00**	**78.82**	**45.20**	**63.33**	**54.84**
Other	-	-	-	-	-	-	-	-	-	-	-	-
Bryozoa	0.36	3.33	0.02	-	-	-	-	-	-	-	-	-
Pisces	-	-	-	4.01	3.33	0.24	-	-	-	-	-	-
Unidentified	8.62	43.33	5.38	8.37	13.33	2.02	16.76	33.33	10.67	2.10	20.00	0.80

Table A8: The relative importance of different prey taxa found in the stomach contents of *R.holubi* caught in the four different habitat types in autumn. The four most important prey items per prey category (excluding 'unidentified') are shown in bold. %*V*= percentage volume, %*F* = percentage frequency of occurrence, %RI = percentage ranking index

Prey item	Mud flats (n=120)			Sand flats (n=120)			Salt marsh (n=120)			*Seagrass* (n=120)		
	%V	%F	%RI	%V	%F	%RI	%V	%F	%RI	%V	%F	%RI
Annelida												
Polychaeta	5.3	6.67	**4.72**	-	-	-	0.70	3.33	0.04	-	-	-
Arthropoda												
Anomura	**5.3**	6.6	0.73	**8.82**	**13.33**	**2.80**	-	-	-	**14.69**	**16.67**	**6.68**
Amphipoda	4.72	**23.33**	2.29	-	-	-	0.06	3.33	0.01	3.69	3.33	0.34
Brachyura	0.2	3.33	0.01	**9.76**	10.00	**2.32**	**7.79**	**16.67**	**2.04**	**9.52**	**20.00**	**5.19**
Copepoda	**20.52**	**43.33**	**18.47**	0.06	3.33	0.01	-	-	-	-	-	-
Mysida	1.5	6.67	0.14	-	-	-	-	-	-	1.23	3.33	0.11
Isopoda	-	-	-	-	-	-	-	-	-	-	-	-
Insecta	-	-	-	-	-	-	0.01	3.33	0.00	-	-	-
Palaemonoidea	-	-	-	0.48	3.33	0.04	2.07	6.67	0.22	-	-	-
Penaeidae	-	-	-	0.47	3.33	0.3	-	-	-	-	-	-
Mollusca												
Bivalvia	-	-	-	-	-	-	-	-	-	-	-	-
Gastropoda	2.25	3.33	0.16	2.64	**13.33**	0.84	**0.89**	**20.00**	**0.28**	1.04	6.67	0.19
Plantae												
Red filamentous algae	**27.48**	**63.33**	**36.16**	**30.25**	**40.00**	**28.76**	**25.69**	**53.33**	**21.52**	**11.21**	**33.33**	**10.20**
Seagrass	**37.59**	**53.33**	**41.66**	**36.91**	**66.67**	**58.48**	**58.8**	**80.00**	**73.91**	**40.26**	**56.67**	**62.25**
Other												
Bryozoa	-	-	-	-	-	-	-	-	-	-	-	-
Pisces	-	-	-	-	-	-	-	-	-	-	-	-
Unidentified	0.91	20.00	0.38	10.61	26.67	6.72	3.88	33.33	2.03	18.37	30.00	15.04

Table A9: The relative importance of different prey taxa found in the stomach contents of *R.holubi* caught in the four different habitat types in winter. The four most important prey items per prey category (excluding 'unidentified') are shown in bold. %*V*= percentage volume, %*F* = percentage frequency of occurrence, %RI = percentage ranking index.

Prey Item	Mud flats (n=120)			Sand flats (n=120)			Salt marsh (n=120)			*Seagrass* (n=120)		
	%V	%F	%RI	%V	%F	%RI	%V	%F	%RI	%V	%F	%RI
Annelida												
Polychaeta	-	-	-	1.9	6.67	0.32	-	-	-	-	-	-
Arthropoda												
Anomura	-	-	-	-	-	-	3.31	6.67	0.38	-	-	-
Amphipoda	0.03	10.00	0.01	-	-	-				0.91	13.33	0.18
Brachyura				-	-	-	10.22	13.33	2.36	1.92	3.33	0.09
Copepoda	0.14	3.33	0.01	-	-	-	-	-	-	-	-	-
Mysida	-	-	-	-	-	-	-	-	-	-	-	-
Isopoda	-	-	-	-	-	-	-	-	-	-	-	-
Insecta	-	-	-	-	-	-	-	-	-	-	-	-
Palaemo-noidea	-	-	-	-	-	-	-	-	-	-	-	-
Penaeidae	-	-	-	-	-	-	-	-	-	1.92	3.33	0.09
Mollusca												
Bivalvia	-	-	-	-	-	-	-	-	-	-	-	-
Gastropoda	0.01	3.33	0.01	**20.06**	**36.67**	**18.59**	3.51	20.00	1.22	1.11	16.67	0.27
Plantae												
Red filamentous algae	**70.43**	**93.33**	**80.00**	**19.28**	**33.33**	**16.25**	**21.80**	**66.67**	**25.33**	**84.28**	**80.00**	**96.8**
Seagrass	**28.98**	**56.66**	**19.9**	**15.86**	**30.00**	**12.03**	**52.61**	**73.33**	**67.22**	**8.02**	**16.67**	**1.92**
Other												
Bryozoa	-	-	-	**41.38**	**50.00**	**52.30**	-	-	-	-	-	-
Pisces	-	-	-	-	-	-	-	-	-	-	-	-
Unidentified	0.38	3.33	0.02	1.51	13.33	0.51	8.54	23.33	3.47	1.82	23.33	0.61