Food resources available to juvenile fish (particularly *Rhabdosargus holubi*) in seagrass beds of the Swartkops Estuary

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Abstract

Estuarine habitats are critical nursery areas for many species of marine fishes during their early life stages in terms of food availability and structural complexity, which increases survival and growth rates. The Cape stumpnose *Rhabdosargus holubi* is one of the most abundant marine fish species found in estuaries along the south-east coast and depends on estuaries as exclusive nursery habitats. This study aimed to identify the epiphytic diatom and macrofauna food resources available to *R. holubi* in the seagrass *Zostera capensis* habitat of the Swartkops Estuary. This habitat has been identified as a core nursery area for this species through a combination of greater abundance, higher growth and survival of juveniles. This study compared what was found in the seagrass habitat and assessed whether this habitat provided for the prey resources most commonly observed in the diet of *R. holubi*. In order to identify the composition of the epiphytic diatom and macrofaunal communities associated with *Z. capensis*, epiphytic diatoms were scraped from seagrass blades, epibionts were counted in the top 20 cm distal section of seagrass blades and infauna were sampled from the sediment of seagrass beds using a van Veen grab. Samples were collected at sites in the lower reaches of the estuary during winter, spring and summer in order to identify how these communities changed temporally. The abundance of epiphytic diatoms differed significantly between seasons (ANOSIM; $R = 0.6$, $p = 0.001$), with diversity (Shannon-Wiener Index: H') showing no differences between seasons ($F_{(2,11)} = 2.97$, $p = 0.09$). The abundance of epibionts differed significantly between seasons (ANOSIM; $R = 0.62$, $p = 0.001$), with diversity showing no differences between seasons ($H = 3.23$, $df = 2$, $p = 0.2$). The abundance of mobile epifauna did not differ between seasons (ANOSIM; $R = 0.06$, $p = 0.28$), with diversity also showing no differences ($H = 1.04$, $df = 2$, $p = 0.59$). The abundance of sediment infauna differed significantly between seasons (ANOSIM; $R = 0.51$, $p = 0.004$), with diversity showing no differences between seasons ($H = 0.27$, $df = 2$, $p = 0.88$). The differences in abundance and diversity were most likely attributed to a number of interacting factors and processes including environmental variables, micro-habitat characteristics and grazing. Diatom species that were identified in the diet of *R. holubi* and occurred on seagrass blades in this study included *Grammatophora*, *Cocconeis*, *Licmophora*, *Navicula* and *Nitzschia* species. Macrofaunal species that were identified in the diet of *R. holubi* and occurred on seagrass blades and in the sediment of seagrass beds in this study included bivalves, polychaetes, isopods, gastropods such as *Nassarius* sp. and *Assiminea* sp., brachyurans such as *Hymenosoma orbiculare* in addition to *Palaemon* shrimps. Seagrass beds have been identified as important nursery habitats as they support an abundant and diverse fish and invertebrate community compared to unvegetated habitats. *Zostera capensis* is listed as endangered and the total area in South Africa is declining as a result of coastal development, habitat destruction, eutrophication and dredging. As *Z. capensis* is a keystone species in the coastal environment, there is a need to protect this habitat to ensure the provision of services including nursery and foraging grounds for estuarine-dependent fishes such as *R. holubi*.

Keywords: food availability, juvenile marine fish, nursery area, diatoms, invertebrates, resource conditions

Declaration

I declare that "**The diversity and abundance of epiphytic diatoms and macrofauna associated with seagrass *Zostera capensis* in the lower reaches of the Swartkops Estuary**" is my own work, that it has not been previously submitted for any degree or examination at any other university, and that all sources I have used or quoted have been indicated and acknowledged by complete references.

Signature: KBraun Date: 23 March 2023

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Chapter 1: General introduction

Estuaries have long been considered as nurseries for invertebrates and fishes. Recent focus has shifted towards examining specific habitats within estuaries as nurseries because they support greater densities of juvenile fish and invertebrates (Whitfield 2017; Whitfield 2020). Most studies on the nursery-role concept indicated that the density of fish and invertebrates were greater in vegetated than in unvegetated habitats (Able 1999; Minello 1999; Castellanos and Rozas 2001; Stunz et al. 2002). Estuarine nursery habitats including seagrass meadows, mangrove forests and salt marshes provide structurally complex habitats, which increases the survival of estuarine-dependent fish and invertebrates, by providing protection from predators and substrate for food resources (Jenkins and Wheatley 1998; Heck et al. 2003; Bloomfield and Gillanders 2005; Schaffler et al. 2013).

Beck et al. (2001) defined a habitat as a nursery for juveniles of a particular species if it contributes a greater than average biomass of juveniles per unit area to adult populations relative to other habitats in which juveniles occur. This occurs through any combination of four factors, including (1) higher density, (2) higher growth, (3) survival of juveniles and (4) movement to adult habitats. The nursery-role hypothesis focuses on a particular set of life history strategies in which a species must at least have some separation between juvenile and adult habitats to be considered to have nursery habitats (Beck et al. 2001).

Seagrass meadows are well known for their nursery function since several studies have reported higher densities of juveniles in seagrass compared to adjacent unvegetated areas (Orth and van Montfrans 1987; Heck et al. 1997; Heck et al. 2003, Leslie et al. 2017). Omnivorous and herbivorous fish species have appeared to be proportionally more abundant in seagrass than in sand and mud flats (Whitfield et al. 2018). In a detailed study of Cape stumpnose *Rhabdosargus holubi* habitat utilisation in the Bushmans Estuary, Leslie et al. (2017) showed that habitat complexity indices were higher in seagrass *Zostera capensis* than in salt marsh grass *Spartina maritima*, and that the abundance of *R. holubi* was significantly greater in the seagrass habitat. Structurally complex habitats decrease predation risk as individuals can hide within plant structures, making them difficult to be detected and captured by predators (Whitfield 1984).

Seagrass occurs in intertidal and subtidal regions of the lower and middle reaches of predominantly open estuaries in South Africa but can be found in temporarily closed estuaries when conditions are saline (Talbot and Bate 1986; Whitfield 2019). These regions can be dominated by *Z. capensis* which has a wide distribution across South Africa occurring in 62 estuaries, with large subpopulations occurring in the Knysna, Berg, Olifants, Langebaan, Keurbooms, Bushmans and Swartkops estuaries (Adams 2016). The Berg (206 ha) and Knysna (238 ha) estuaries have the largest stable *Z. capensis* beds (Adams 2016). Coastal development and human disturbance such as boating and bait digging have reduced the area cover and biomass of *Z. capensis*, which has a cascading effect on higher trophic levels (Adams 2016). Eutrophication is a growing threat in South African estuaries as nutrient enrichment leads to the growth of nuisance macroalgal populations, which shade and outcompete *Z. capensis* for light and nutrients, and further decreases overall species diversity of associated biota (Adams 2016). *Zostera capensis* now qualifies as endangered in South Africa with improved protection being required for this species (Adams and van der Colff 2018).

The estuarine-dependent *R. holubi* is one of the most dominant and well-studied marine fish species occurring in estuaries along the southern and eastern coasts of South Africa and is strongly associated with vegetated habitats (Whitfield 1984; Whitfield et al. 2018). This species migrates into estuaries as postflexion larvae from the sea during late winter, spring and early summer where they spend at least the first year of their life moving between lower riverine and estuarine habitats as juveniles, before leaving the estuary to the marine environment for maturation and spawning (Wallace et al. 1984; Carassou et al. 2016). Higher temperatures and rich food supply in estuaries, favours the rapid growth of this species (Blaber 1973; de Wet and Marais 1990). The feeding of *R. holubi* changes as they grow, with juveniles being described as omnivorous and feeding on aquatic macrophytes and filamentous algae together with associated epiphytes and epifauna in addition to invertebrates (Blaber 1973; de Wet and Marais 1990; Carassou et al. 2016). *Rhabdosargus holubi* do not appear to assimilate plant material due to the lack of cellulase but digest the epiphytic diatoms covering the leaves of macrophytes (Blaber 1974b; Sheppard et al. 2012).

The aim of this study is to identify food resource availability in terms of epiphytic diatoms and macrofauna associated with *Z. capensis* in the lower reaches of the Swartkops Estuary. This study will compare what is found in the seagrass habitat to food resources found in the diet of *R. holubi* and assess whether this habitat is suitable and useful for *R. holubi* in terms of known prey resources, addressing the following questions:

1) What are the food resources available to *R. holubi* in the seagrass habitat of the Swartkops Estuary?

2) How does food availability change temporally?

3) Is the seagrass habitat of the Swartkops Estuary a suitable nursery habitat for *R. holubi* in terms of food availability?

An assessment of food resources available to *R. holubi* such as macroinvertebrates and epiphytic diatoms within seagrass beds would help in understanding what important food resources are available for juvenile fishes within this habitat in the Swartkops Estuary.

Dissertation outline

Following this general introduction (**Chapter 1** – above), this dissertation is structured in the format of two standalone data chapters presented to be suitable for submission as peer-reviewed scientific publications. As such, there is some necessary overlap between chapters, although this is kept to a minimum where possible, and the general introduction chapter has been kept succinct in favour of more specific, detailed background information being presenting in the data chapters.

A literature review (**Chapter 2**) elaborates on the information above, followed by a detailed overview of the study site (**Chapter 3**).

The first results presented (**Chapter 4**) directly addresses how the epiphytic diatom assemblages associated with *Z. capensis* is structured within the Swartkops Estuary, as well as how this changes temporally, while the second set of results (**Chapter 5**) examines how benthic infauna adjacent to the *Z. capensis* beds as well as epibionts and mobile epifauna within the seagrass vary temporally.

Finally, this dissertation is concluded (**Chapter 6**) by a synthesis of the results collected to address the third research question of whether this estuarine habitat appears suitable as a nursery region for *R. holubi* in terms of available food resources.

Chapter 2: Literature review

Estuaries

Estuaries are defined as semi-enclosed coastal bodies of water formed where freshwater from rivers runs out to sea, despite the mouths of some estuaries being periodically closed off from the sea (Pritchard 1967; Driver et al. 2012; van Niekerk et al. 2020). Estuaries are physico-chemically variable due to the constant mixing between freshwater and seawater and as a result of this variation, estuaries possess a unique salinity profile and characteristic biota (Elliott and Whitfield 2011).

Freshwater inflow is one of the main sources of nutrients for an estuary which fuels estuarine productivity (Scharler and Baird 2003). The organisms within estuaries are able to convert nutrients into organic material through autochthonous production by phytoplankton, macrophytes, macroalgae and benthic microalgae (Elliott and Whitfield 2011). Estuaries also receive large amounts of organic material from riverine primary producers, the marine environment and anthropogenic waste (Abrantes and Sheaves 2010; Howe and Simenstad 2011). The ability of an estuary to convert nutrients to organic material is determined primarily by the residence time and light regime (Elliott and Whitfield 2011). A short residence time (caused by a high tidal range and/or freshwater flushing) and high turbidity will result in the nutrients entering the estuary being exported to the adjacent coast before being utilised by the plants within the estuary. Conversely, an increased residence time favours autochthonous production and can even result in a phytoplankton or flagellate bloom to develop within an estuary (Hilmer and Bate 1990).

The outwelling hypothesis proposed by Odum (1980), states that estuarine systems produce more material (particulate and dissolved carbon and inorganic nutrients) that can be utilised or degraded within the system, that this excess material becomes exported to the marine environment, where it supports nearshore food webs (Dame et al. 1986; Winter et al. 1996).

Ecosystem services

Estuaries are rich and productive systems that provide a wide range of ecosystem services (contributions that ecosystems make to human well-being) to society, they are therefore, considered to be one of the most valuable types of ecosystems on earth (Nagelkerken et al. 2015).

Estuaries make contributions to livelihoods, marine fishery values, the amelioration of climate change damages in addition to property values, tourism and recreation. In South Africa, estuaries contribute to livelihoods by providing a wide range of natural resources including fish, invertebrates, medicinal plants, reeds and wood (from mangrove forests) which are harvested for nutrition, energy and raw materials (Turpie and Letley 2019). Estuaries contribute to marine fishery values by providing nursery areas for many fish and invertebrate species that are harvested for commercial or recreational purposes (Whitfield 1994). Estuarine habitats including mangroves, seagrasses and salt marshes are highly productive systems which can sequester carbon at a rapid rate (Barbier et al. 2011; Beaumont et al. 2014). ‘Blue carbon’ is a term that is used to describe the carbon that is found in these three ecosystems (mangroves, seagrass and salt marsh) (Siikamäki et al. 2012). The carbon that is sequestered by these habitats during photosynthesis is moved from the short-term carbon cycle (10-100 years) to the long-term carbon cycle (1000 years) and is continuously buried as slowly decaying biomass (Barbier et al. 2011).

Estuaries contribute to property values as people are willing to pay a premium to live near them (having access to or being able to view them) (Turpie and Clark 2007). Estuaries were estimated to add R20 billion to the value of coastal property in South Africa with an estimated R609 million in terms of direct contribution to GDP (van Niekerk et al. 2019). Tourism activities can be centred around estuaries in South Africa as they offer a host of recreational activities including boating, fishing, swimming, paddling and bird watching (Turpie and Clark 2007). Out of R10.20 billion per annum attributable to recreational and tourism activities in coastal areas surrounding South African estuaries, R2.40 billion per annum was attributed to the estuaries themselves, with larger and more developed estuaries including Knysna, Langebaan, St Lucia, Breede, Gouritz, Groot Berg and Keurbooms having higher values (van Niekerk and Turpie 2019).

Estuarine habitat complexity

Estuarine habitats are transitional tidal zones occurring between land and sea, which are occupied by algae, submerged macrophytes and emergent aquatic and terrestrial species (Lubke and van Wijk 1988). The habitats recorded in South African estuaries include the open surface water area, sand and mud banks, macroalgae, submerged macrophytes, salt marsh, reeds and sedges, mangroves, rocks and swamp forests (Adams et al. 2016; van Niekerk and Turpie 2019). Submerged macrophytes in estuaries are important foraging and refugia habitats for a wide variety of fish and invertebrates, with their structural complexity providing protection from predators, reducing water turbulence and creating diverse microhabitats (Sánchez-Botero et al. 2011; Leslie et al. 2017; Whitfield 2019). Submerged macrophytes also play an essential role in biogeochemical processes including oxygenating the water column through photosynthesis (during the growing season), improving water clarity in addition to nutrient trapping and recycling (van Niekerk et al. 2019).

There are three main types of submerged macrophytes occurring in estuaries depending on the salinity and estuarine type, these include the eelgrass *Z. capensis*, *Ruppia cirrhosa* and *Potamogeton pectinatus* (Whitfield 2019). *Zostera capensis* is abundant in predominantly open estuaries where euryhaline salinities are prevalent, *R. cirrhosa* occurs in both predominantly open and small/large temporarily closed estuaries where salinities range from mesohaline to euhaline and *P. pectinatus* occurs in small/large temporarily closed estuaries where oligohaline salinities prevail (Whitfield 2019). Seagrass meadows were identified as critical nursery habitats for juvenile fish by Heck et al. (2003), who reviewed literature relating to the nursery-role hypothesis. A vast majority of studies confirmed that the growth, survival and abundance of fish species were significantly greater in seagrass meadows compared to that of unstructured habitats such as sand and mud flats. Studies have also found that nursery species preferred clearer water in seagrass meadows than turbid water in unvegetated sandy areas; however, it was argued that this was due to the protection provided by the structural complexity of seagrass instead of reduced visibility due to turbidity (Perry et al. 2018).

Seagrass and epiphytes

Zostera capensis or Cape dwarf-eelgrass is the dominant seagrass occurring in sheltered estuaries along the nearly 3000 km South African coastline, with its protected meristems, strong root system and flexible leaves allowing it to grow where there are strong tidal currents in addition to being able to tolerate periods of exposure and desiccation (Barnabas 1977; den Hartog and Kuo 2006; Adams 2016). The strap-like leaves of *Z. capensis* is able to withstand tidal currents better than the branching leaf and stem morphology of *Ruppia* spp., explaining its dominance in the intertidal zone of predominantly open estuaries (Adams 2016). Overlapping leaves during periods of exposure (desiccation) helps to reduce water loss due to *Z. capensis* having no physiological barrier preventing water loss (Adams 2016). *Zostera capensis* occurs in 62 out of more than 290 estuaries in South Africa, with Knysna Estuarine Bay having the largest distribution of this species (353 ha) (Adams and van der Colff 2018). Other important large subpopulations of *Z. capensis* occur in the Kosi, uMhlathuze, Qora, Keiskamma, Kariega, Bushmans, Swartkops, Kromme, Keurbooms, Langebaan, Berg and Olifants estuaries, showing varying changes in coverage over time (Adams and van der Colff 2018).

Zostera capensis is now assessed as endangered in South Africa due to coastal development, habitat destruction in addition to its continued decline in response to pressures such as eutrophication and dredging (Cyrus et al. 2008; Adams and van der Colff 2018). Human disturbance as a result of bait digging and boating reduces cover and biomass (Pillay et al. 2010). Improved protection is required for this nationally important eelgrass species, especially in the Knysna Estuarine Bay which has the largest distribution of *Z. capensis* in South Africa.

A greater abundance of juvenile fish and invertebrate species have been associated with seagrass beds compared to unvegetated benthic habitats (Hanekom and Baird 1984; Whitfield 1989; Edworthy and Strydom 2016) as they provide a protective habitat and both a direct and indirect (through epiphytes) source of food (Hemminga and Duarte 2000). Seagrass leaves and stems provide the primary substratum for the attachment of epiphytes in seagrass beds (Moncreiff et al. 1992; Michael et al. 2008). Epiphytes are defined as plants living on the exterior of other plants and includes diatoms, cyanobacteria, macroalgae and encrusting algae (van Montfrans et al. 1984). Attachment to seagrass can enhance access to irradiance for photosynthetic algae, with epiphyte productivity contributing to the overall productivity of seagrass meadows (Jernakoff et al. 1996; Michael et al. 2008). Structural factors including seagrass leaf area and turnover rate, influence the space and time for epiphyte colonisation and growth (Frankovich and Fourqurean 1997). Physicochemical factors including nutrient availability, temperature, salinity in addition to light availability and quality, influence epiphyte loads (Frankovich and Fourqurean 1997). Of these factors, increased nutrient availability and decreased seagrass leaf turnover rates, have been identified as factors leading to increased epiphyte loads in seagrass beds (Borum 1985; Twilley et al. 1985; Tomasko and Lapointe 1991). Epiphyte abundance generally increases as a function of nutrient availability (Borum 1985).

Seascape connectivity

Coastal fish are capable of utilising multiple habitats during different life stages and connect habitats through daily movement, larval dispersal and ontogenetic migrations (Gillanders et al. 2003; Nagelkerken 2007; Perry et al. 2018). There has, therefore, been a shift in examining connectivity between habitats and the concept of a broader seascape nursery (Nagelkerken et al. 2015; Olds et al. 2016; Perry et al. 2018). The seascape nursery consists of connected mosaics of habitat patches that are functionally connected, instead of viewing the nursery function of habitats in isolation (Nagelkerken et al. 2015). Hotspots of high animal abundances represents the core area of a habitat mosaic, which is spatially constrained by the home ranges of its occupants (Nagelkerken et al. 2015).

Figure 2.1 is an example of a microtidal seascape which consists of a variety of habitat types, where fish settle in first-encountered non-core habitats including coral rubble areas along the edges of tidal channels or at bay mouths, subsequently progressing to seagrass beds and then to mangroves, and finally occupying hard-bottom patch reefs or rocky areas before moving to offshore reefs (example from Nagelkerken et al. 2015 and Grol et al. 2011). During rubble and seagrass occupancy, small juveniles feed and take shelter in the same habitat to reduce predation, however, at larger sizes they use mangroves and patch reefs for shelter and show a diel or tidal migration to nearby seagrass beds to feed (Verweij et al. 2006). During these movements individuals pass through secondary habitats, including algal beds and sand patches, as they move from one feeding patch to another. These secondary habitats do not play an important role in feeding or shelter, but form part of their home range (showed by concentric circles in Figure 2.1). In this example, seagrass beds would be identified as the core nursery habitat as they provide the largest overall contribution to adult populations (Verweij et al. 2006). The seascape nursery concept suggests that (1) temporary settlement areas should be conserved as without these, there is no recruitment to nursery habitats; (2) there are principle areas (which are constrained by animal home ranges), within the seascape, that attract higher densities of mobile organisms and are more productive than other areas, providing a management tool to prioritize areas of conservation; (3) successive life-stage habitats should be conserved as impacts on one habitat affects productivity in other habitats that are occupied during later life stages; (4) without conserving migration routes that connect different animal hotspots during ontogeny or facilitate movement from nursery areas to offshore populations, nurseries could shift from acting as sources to becoming juvenile sinks (Nagelkerken et al. 2015).

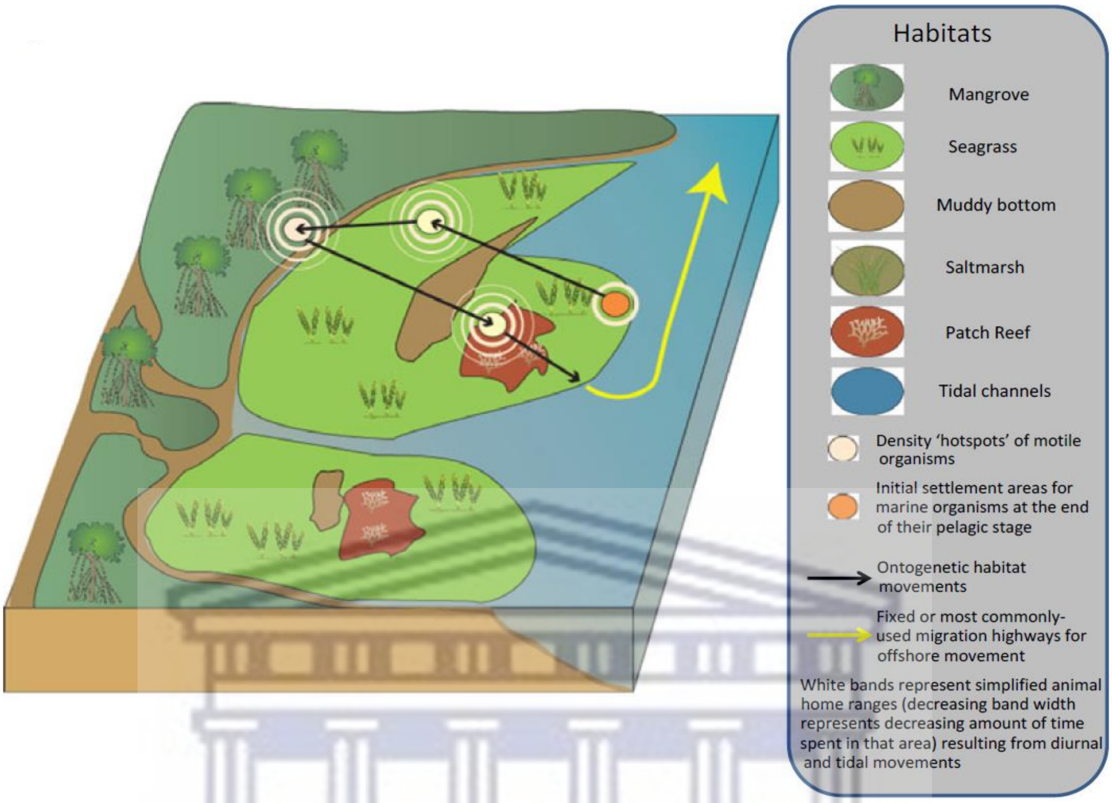

Figure 2.1: Example of a seascape nursery consisting of a variety of habitat types that are connected through diel and ontogenetic movements. Image acquired from Nagelkerken et al. (2015)

The nature of habitat connectivity in shallow-water coastal seascapes was examined in a study by Perry et al. (2018), which compared fish assemblage composition in three adjacent shallow-water habitats including seagrass meadows, rocky bottoms covered by macroalgae in addition to unvegetated areas, on the Swedish Skagerrak coast. This study found similar species composition in the adjoining habitats with neither the number of species nor species diversity differing among these three habitats. This implies a degree of connectivity between habitats and indicates that the fish community is similar in adjacent habitats of temperate shallow-water seascapes. As previously stated, structurally complex habitats including seagrass meadows and macroalgal beds, had significantly higher abundances of fish than unvegetated areas. The connectivity of shallow-water habitats is essential for maintaining healthy fish communities.

Threats to estuaries

Anthropogenic activities have significantly altered estuarine systems due to accelerated population growth and urban development in the coastal zone (Kennish 2002). Urbanisation has led to increased stormwater volumes running off paved areas and being directly discharged into aquatic ecosystems, where it harms the water quality and ecosystem health status of these areas (Adams et al. 2019). Estuaries receive a large amount of nutrients from allochthonous anthropogenic sources including municipal and industrial wastewater, sewer overflows, farmlands and defective septic systems (Kennish 2002). The pollution of estuarine systems is attributed to anthropogenic nutrient enrichment and organic carbon loading (sewerage), which results in the eutrophication of estuarine waters (Kennish 2002). The influx of nutrients including nitrogen and phosphorus from anthropogenic activities, stimulates the production of phytoplankton, which settles to the bottom of the estuary and results in hypoxia through bacterial decomposition pathways (Capriulo et al. 2002). This further leads to nuisance algal blooms, the mortality of pelagic and benthic species, shading effects in addition to reduced biodiversity (Kennish 2002). Estuaries are often exploited due to their abundant resources and economic importance, with the overexploitation of recreationally and commercially important finfish and shellfish populations, depleting resources beyond sustainability limits and further having a direct effect on humans (Kennish 2002).

Human et al. (2016) determined the impact of a large opportunistic macroalgal bloom, with *Ulva lactuca* as the dominant species, on *Z. capensis* beds in Ashmead Channel in the Knysna Estuary. The results showed a decrease in the percentage cover of *Z. capensis*, which had been displaced by the algal bloom. The canopy height of the algal bloom reduces the light that is able to reach the underlying vegetation, which results in both benthic algae and submerged macrophytes not being able to photosynthesize. Other studies have also observed a decrease in *Zostera* abundance with an increase in *Ulva* abundance (Berglund et al. 2003; Burkholder et al. 2007). It was stated that the anoxic sediment itself may have also caused the decline of *Z. capensis* in Ashmead Channel. The study by Human et al. (2016) recommended that *U. lactuca* be harvested for compost and fertilizer due to the high tissue N and P and low metal concentrations, this would enable the recovery of *Z. capensis* and limit the impact of the algal bloom on seagrasses, which would also prevent further losses. Barnes (2018) repeated a 2011 survey of then seagrass macrobenthic assemblages in the Steenbok Channel of the Knysna Estuary, following a green tide in the late austral summer of 2015 that left bare muddy sediment. This green tide killed off an extensive area of seagrass, with *Ulva* blanketing many of the intertidal seagrass beds of the systems outer marine embayment. The study showed that after seagrass loss, intertidal macrofauna became significantly less speciose and spatially and systematically more uniform, but more abundant. The faunal change was attributed to the loss of seagrass cover, which was supported by the similarity of the 2018 fauna, to that occurring in other pre-existing bare sediment in the estuary (Barnes and Barnes 2014).

A rapidly growing demand for more freshwater, not only to meet the domestic demands of increased numbers of people in the coastal region, but also to meet the requirements of agricultural and industrial interests, is a major threat to estuaries worldwide (Rosenberg et al. 2000; Kennish 2002). Runoff river freshwater abstraction, small farm dams and large impoundments are primarily responsible for decreasing the overall quantity of freshwater that reaches South African estuaries (Whitfield and Wooldridge 1994). Other activities contributing to flow modification in South African estuaries, that are responsible for increased freshwater inflow to systems that historically received a lower inflow, include inter-basin transfer schemes, wastewater treatment works and increased runoff from “hardened” catchments (e.g. road networks) (Nirupama and Simonovic 2007). Changes in freshwater inflow into estuaries, impacts the ecological functioning of these systems, for example, changes in mouth closure frequencies have been directly linked to decreased freshwater inflow as seen in the Kobonqaba and Uilkraals estuaries, these systems were historically predominantly open, but closed for the first time in 2010 (van Niekerk et al. 2013). It is estimated that more than a third of the freshwater that used to reach the coast, is now abstracted from South Africa estuaries across biogeographical regions, with present inflows down from 36 900 to 24 800 x10^6 m^3 /a (van Niekerk et al. 2019).

Land-use changes and development affects the health and ecological functioning of estuaries, with the most severe land-use changes and developments affecting South African estuaries being road bridges and mining, port and marina developments together with their associated dredging activities (van Niekerk et al. 2013). Other significant impacts include changes to erosion/depositional cycles, changes to flow velocity and circulation patterns, direct habitat loss during construction, smothering of submerged habitats by excessive sedimentation in addition to contamination and associated poor water quality (Morant and Quinn 1999; Whitfield et al. 2012). These physical changes negatively affect biota and can lead to local extinctions, changes in population size or biomass, changes in community composition and structure in addition to changes in the ratios of generalist to specialist biota as well as life-history patterns (Vorwerk et al. 2003; Levin and Stunz 2005; van Niekerk et al. 2013). Habitat degradation can make an estuary prone to invasion by more generalist alien species, such as invasive *Spartina alterniflora* (North America cordgrass) in addition to *Tarebia granifera* (Asian mollusc) (Appleton et al. 2009; Adams et al. 2012).

Oceanic sea level rise (SLR) is predicted to reach between 0.61 and 1.10 m by 2100 under higher emissions scenarios (Oppenheimer et al. 2019). An increase in sea level rise and extreme weather events, may alter the hydrogeomorphology of estuaries and lead to the loss of important estuarine habitats including salt marsh areas and mangrove forests, which will affect estuarine fish communities in addition to fisheries that target estuary-associated species (Clark 2006).

Swartkops Estuary

Swartkops Estuary is one of the largest estuaries in the Eastern Cape and is ranked 11th out of more than 280 estuaries in South Africa attributed to its size and habitat and biodiversity importance (Emmerson 1985; Turpie et al. 2002). It is an essential nursery habitat for fish with over 80 fish species being identified at this estuary in addition to being the most important breeding site for colonies of coastal birds on the mainland of the Eastern Cape (van Niekerk and Turpie 2012). Swartkops Estuary has the third largest salt marsh area in South Africa, supporting a large meiofauna community as well as consisting of six different plant community types, including inter- and supratidal salt marsh, sedges, reeds, phytoplankton, submerged macrophytes and benthic microalgae (Colloty et al. 2000). The salt marsh community of the Swartkops Estuary has experienced the greatest loss due to this area being used for residential and industrial development (Colloty et al. 2000; Bornman et al. 2016; Adams et al. 2021). **Chapter 3** contains a detailed study site description of Swartkops Estuary.

Microalgae

Masson and Marais (1975) recorded 18 phytoplankton species in the water column and 40 species in the sediment of the Swartkops Estuary with the latter comprising 34 species of naviculoid diatoms, 3 desmid species, 2 chlorophyte species and a single foraminiferan species. Of these, microplankton (>12 µm) is the dominant size group, accounting for more than 60 % of the total production in the Swartkops Estuary (Hilmer et al. 1998).

Diatoms are defined as unicellular algae that occur as single cells or in colonies wherever there is sufficient light and nutrients (Armbrust 2009). Diatoms possess a siliceous cell wall, unique photosynthetic pigments and specific storage products such as oil and chrysolaminarin which distinguishes them amongst the algae (Drum and Gordon 2003; Taylor et al. 2007). Diatoms form a fundamental link between primary and secondary production in aquatic ecosystems due to being integrated into aquatic food webs through selective feeding, accidental ingestion or the breakdown of the host (Ziemann and Wetzel 1980; Gordon et al. 2008). Key natural microhabitats for diatoms include solid substrata, exposed damp sediments in addition to the stems of rooted vegetation. Diatoms are also present in the seston or suspended component of the phytoplankton in addition to man-made objects such as plastic and paper bags (Taylor et al. 2007). Diatoms form the dominant group of epiphytic communities occurring on submerged macrophytes, with epiphytes forming an important linkage in the structure of estuarine food webs (Coleman and Burkholder 1995; Gordon et al. 2008).

In a project to prepare a river water diatom identification database for use in South Africa and to identify the use of diatoms in the assessment of water quality, Bate et al. (2002a) showed that the diatom *Navicula gregaria*, was dominant in the epipelon (grows on fine sediments) at certain sites where the concentrations of Cl^-, EC, Mg^{++}, Na^+, NH_4^+, NO_2+NO_3^-, PO_4^-, SiO2 and SO_4^- were low in the Swartkops River, i.e., it was an indicator of better water quality than when it was present in only small numbers. The epilithic diatom, *Achnanthes minutissima*, was dominant in the cleaner waters of the Swartkops. The diatom *Nitzschia frustulum*, was the second most dominant epilithic diatom which was an indicator of polluted sites on epilithic habitats. Other species that are dominant in the epipelon in the Swartkops River include *Achnanthes exigua*, *Amphora* cf. *luciae*, *Bacillaria paxillifer*, *Diploneis puella*, *Fragilaria elliptica*, *Mastogloia elliptica*, *Nitzschia capitellata*, *Karayevia amoena*, *Gyrosigma prolongatum* var. *closteroides*, *Nitzschia angularis* and *Gyrosigma stompsii* (Bate et al. 2004).

Invertebrates

A total of 122 species of benthic macrofauna was recorded by Baird et al. (1986) in the Swartkops Estuary, with the greatest diversity occurring in the intertidal reaches. Mudprawns *Upogebia africana*, sandprawns *Kraussillichirus kraussi*, grapsoid crabs *Cleistostoma* spp. and Pencilbait *Solen* spp. are the most common invertebrates (Hanekom et al. 1988). Other abundant macrobenthic species include clams *Dosinia hepatica* and *Salmacoma litoralis*, the tongue worm *Listriolobus capensis*, tapeworm *Polybrachiorynchus dayi* as well as cracker shrimp *Alpheus lobidens*, which are also found in the middle reaches. Dominant macrobenthic fauna in the salt marsh habitat includes two detritivorous crab species *Parasesarma catenatum* and *Danielella edwardsii* in addition to the gastropod *Assiminea* sp. (Baird et al. 1986; Els 1982). The mud crab *Scylla serrata* occupies intertidal burrows in the middle to upper reaches.

Hanekom et al. (1988) estimated the numbers and standing biomass of the 10 most abundant macrobenthic species in the estuary. *Upogebia africana* was the dominant species inhabiting the muddy intertidal areas of the lower reaches (82 % of the total recorded standing biomass). *Kraussillichirus kraussi* was the second most important species (10 % of the total recorded biomass) inhabiting the inter- and subtidal regions of the mouth and upper reaches. This species was rarely recorded from the muddy intertidal areas of the lower reaches most likely due to competition with *Upogebia africana*. *Solen capensis* was the third most dominant species (3 % of the total recorded biomass) inhabiting the sandier areas of the lower reaches and together with *Solen cylindraceus* (1 % of the total recorded biomass) comprised the dominant bivalves. Other abundant macrobenthic species included *Paratylodiplax algoensis, Alpheus lobidens, Dosinia hepatica*, *Danielella edwardsii*, *Listriolobus capensis* and *Salmacoma litoralis*.

Mclachlan and Grindley (1974) recognized four major macrofauna communities in the Swartkops Estuary, each one dominating one reach of the estuary with a minor community dominating the silty heads of creeks. These included a *Kraussillichirus* community dominated by *Kraussillichirus kraussi* in the mouth area, an *Upogebia* community dominated by *Upogebia africana* in the intertidal muds of the lower reaches, a bivalve community dominated by *Dosinia hepatica*, *Salmacoma litoralis*, *Solen corneus* and *Eumarcia paupercula* in the middle reaches. The sands of the upper reaches were also dominated by *Kraussillichirus kraussi*. The creek community was dominated by the shrimp *Palaemon pacificus*, the cracker shrimp *Alpheus lobidens* and two small crabs including *Danielella edwardsii* and *Paratylodiplax algoensis*.

Over 50 species of zooplankton have been identified in the Swartkops Estuary, with copepods and mysids dominating (Wooldridge and Mellville-Smith 1979).

Fish

Over 80 fish species have been identified in the Swartkops Estuary (Scharler et al. 1997). The seagrass habitat is an important nursery area for juvenile fish with the Cape silverside *Atherina breviceps*, estuarine round-herring *Gilchristella aestuaria*, Cape Stumpnose *Rhabdosargus holubi* and white seabream *Diplodus sargus*, dominating this habitat in the estuary (Enviro-Fish Africa 2009).

Coastal marine fish utilise multiple habitats at different life stages in search of food and shelter from predation (Gillanders et al. 2003). Fishes associated with South African estuaries may be divided into four broad categories according to origin and spawning habits, these include marine, estuarine, freshwater and catadromous species (Whitfield 2019). Marine species usually breed at sea with some taxa recorded spawning in the mouth region of certain estuaries. The juveniles of most marine species utilise estuarine habitats as nursery areas. Estuarine species generally spawn within the estuarine system with several taxa recorded breeding both in the marine and freshwater environment. The entire life cycle of some of these fishes occurs in estuaries, while other species have part of their life cycle spent in either the sea or adjacent freshwater habitats. Freshwater species usually spawn in rivers, streams, lakes or pans with some species recorded breeding in estuaries (e.g., *Oreochromis mossambicus*). Catadromous species consists of anguillid eels, which spawn at sea, but spend most of their life in river systems (Whitfield 2019).

The marine category comprises three guilds including marine stragglers (species that occur ‘accidentally’ in estuaries) (Figure 2.2a), marine estuarine-opportunist (species that tend to enter estuaries in large numbers at some point in their life cycle, typically during the juvenile phase) (Figure 2.2b), and marine estuarine-dependent species (species that depend on estuaries for survival at a critical stage in their life cycle) (Potter et al. 2015). The estuarine category comprises four guilds, including the solely estuarine guild (species that complete their entire life cycle in estuaries) (Figure 2.2c), the estuarine and marine guild (Figure 2.2d) and estuarine and freshwater guild (Figure 2.2e) (species that contain populations that complete their life cycles in the estuary, but which are also represented by populations in either marine or freshwater environments respectively) in addition to the estuarine migrant guild (species whose postflexion larvae are flushed out to sea and considerable numbers of which survive, return to the estuary as postflexion larvae or early juveniles) (Figure 2.2f) (Potter et al. 2015). The diadromous category comprises anadromous species (diadromous fishes which spend most of their lives at sea and which migrate to freshwater to breed) (Figure 2.2g), catadromous species including anguillid eels (diadromous fishes which spend most of their life in freshwater and which migrate to the sea to breed) (Figure 2.2i), semi-anadromous (a small number of anadromous species whose upstream migration from the sea, does not extend beyond the upper reaches of the estuary) (Figure 2.2h), semi-catadromous (a few catadromous species whose downstream migration to the sea, does not extend beyond the lower estuary) (Figure 2.2j), and amphidromous species (diadromous fish whose migration from freshwater to the sea, or vice versa, is not for the purpose of breeding, but occurs regularly at some other definite stage of the life cycle) (Figure 2.2k) (Potter et al. 2015). The freshwater category comprises the freshwater straggler guild (freshwater species that are usually only found in low numbers in estuaries) (Figure 2.2l) and the freshwater estuarine-opportunist guild (freshwater species that are found regularly in estuaries, generally in moderate numbers) (Figure 2.2m) (Potter et al. 2015).

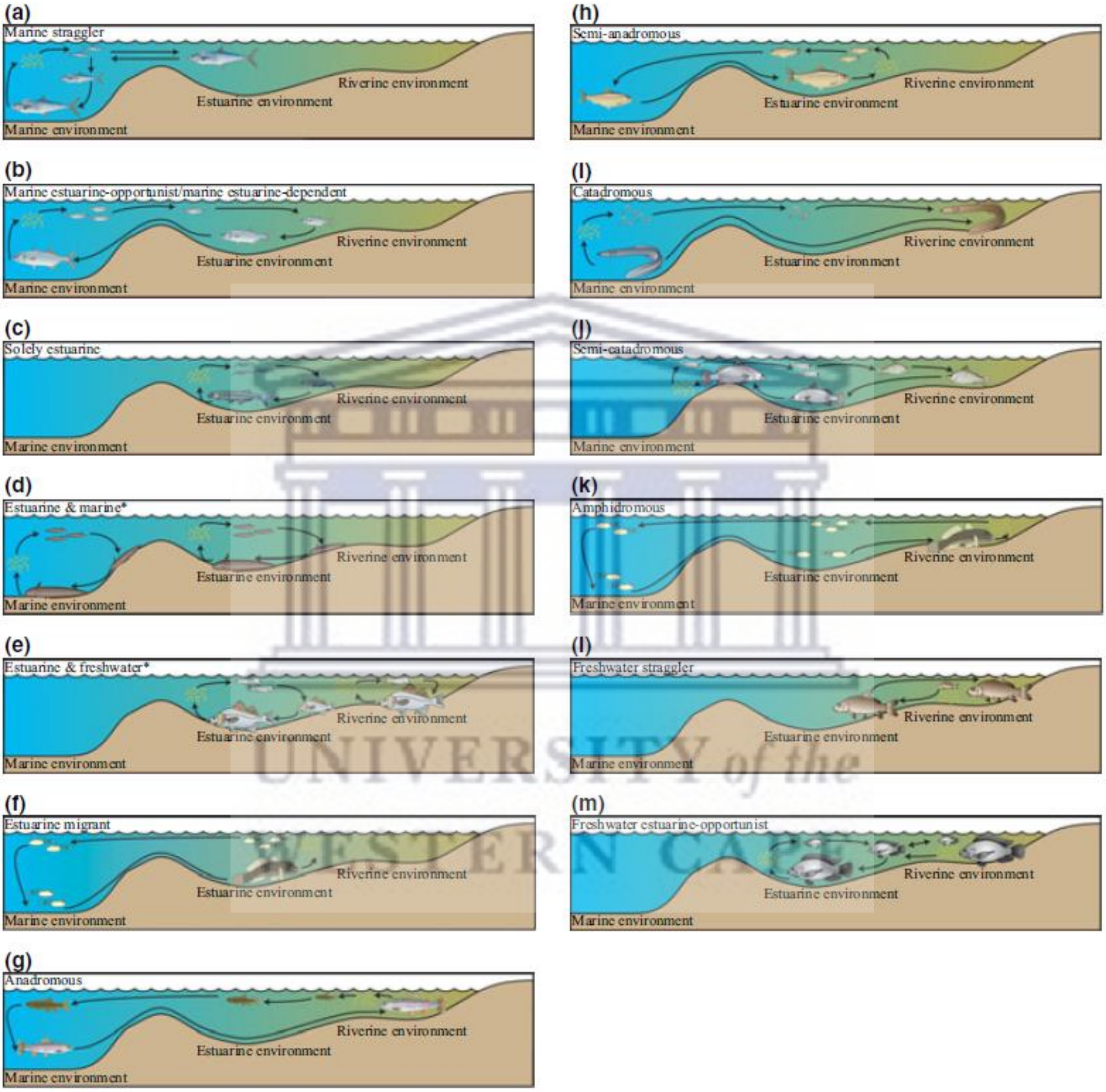

Figure 2.2: Guilds of fishes found in estuaries (*refers only to the estuarine populations of the guild). Image acquired from Potter et al. (2015)

Birds

Swartkops Estuary is the most important breeding site for colonies of coastal birds (including African Black Oystercatcher with up to 14 pairs annually) on the mainland of the Eastern Cape Province (van Niekerk and Turpie 2012) and forms part of a Global Important Bird Area (IBA). The estuary frequently hosts over 4000 birds of over 200 species (Marnewick et al. 2015). Of these, 77 species are waterbirds and three are raptors associated with wetlands including Western Osprey *Pandion haliaetus*, African Fish Eagle *Haliaeetus vocifer* and African Marsh Harrier *Circus ranivorus* (Marnewick et al. 2015). The intertidal mudflats near the river mouth, support the greatest density of birds, with important numbers of Terek Sandpiper *Xenus cinereus*, African Black Oystercatcher *Haematopus moquini*, Ruddy Turnstone *Arenaria interpres* and Common Whimbrel *Numenius phaeopus* (Marnewick et al. 2015). Two islands at Redhouse Salt Pans host the second largest breeding colony of White-breasted Cormorant *Phalacrocorax lucidus* in southern Africa, with a maximum of 224 nests counted (Marnewick et al. 2015). Other regionally important breeding colonies include that of the African Sacred Ibis *Threskiornis aethiopicus* and the Grey-headed Gull *Chroicocephalus cirrocephalus* (with up to 608 nests).

Globally threatened species include Knysna Woodpecker *Campethera notata*, Damara Tern *Sterna balaenarum*, Chestnut-banded Plover *Charadrius pallidus*, African Black Oystercatcher, Martial Eagle *Polemaetus bellicosus*, Cape Cormorant *Phalacrocorax capensis* and Lesser Flamingo *Phoeniconaias minor*. Regionally threatened species include Greater Painted-snipe *Rostratula benghalensis*, Caspian Tern *Hydroprogne caspia*, Half-collared Kingfisher *Alcedo semitorquata*, Roseate Tern *Sterna dougallii*, African Marsh Harrier, Greater Flamingo *Phoenicopterus roseus* in addition to Lanner Falcon *Falco biarmicus* (Marnewick et al. 2015).

Martin and Baird (1987) found seasonal variations in the numbers of both migrant and resident bird species for Swartkops Estuary, over 4000 birds were recorded in the austral summer, whereas less than 1200 birds were counted in winter. Five bird species including Curlew Sandpipers *Calidris ferruginea*, Kelp Gulls *Larus dominicanus*, Common Terns *Sterna hirundo*, Grey Plovers *Pluvialis squatarola* and Whimbrels comprised 74 % of the total number of birds. The majority of birds (92 %) were recorded on the intertidal sand and mud and a positive correlation between bird density and the biomass of a major prey species, *U. africana*, was found.

Threats to the Swartkops Estuary

The provision of ecosystem services by South African estuaries is being threatened by the demand on coastal resources to support societal needs and socio-economic benefits. Key threats to South African estuaries include habitat destruction as a result of low-lying developments, exploitation of fishing resources, flow modification due to water abstraction and increased urban runoff in addition to agricultural and industrial pollution (van Niekerk and Turpie 2012). Microplastic pollution is an emerging threat to South African estuaries with plastic pollution being greatest in metropolitan areas especially where estuaries serve as industrial outlets (Naidoo et al. 2015). Microplastics enter estuaries through anthropogenic waste from urban and industrial activities and are a major threat to marine habitats as these particles can become ingested by invertebrates (bivalves, crustaceans and polychaetes) and vertebrates (fish and seabirds) due to their size. Nutrient input from the Motherwell and Markman canals (stormwater canals draining township and industrial areas respectively) in addition to the Chatty River (draining highly populated township areas) are the main sources of pollution for the Swartkops Estuary. The Motherwell Canal is a major source of nitrogen (in the form of ammonium) to the estuary (Adams et al. 2019). Faecal waste enters the middle reaches of the estuary via the Motherwell Canal due to leaks in the sewer system of the Motherwell Township area.

Management of the Swartkops Estuary

Despite the fact that the biodiversity value of estuaries has been known for a long period of time, there has been a lack of effective estuarine management in South Africa, due to the fact that it did not fit into the mandate of any government department (Retief et al. 2016). Estuaries and the management thereof, now form an integral part of the Integrated Coastal Management Act (Act 24 of 2008; ICM Act). The need for estuarine management plans in South Africa is required in order to mitigate the harmful impacts of anthropogenic activities on estuarine ecosystem services (de Villiers 2016). The aim of estuarine management plans is to prevent potential impacts on the environment, instead of only responding once the impacts have occurred. Integrated and cooperative efforts are required to ensure that future development and activities are managed in order to prevent further damage to these already degraded ecosystems.

The Swartkops Valley and the Aloes Nature Reserves, located immediate north of the Swartkops Estuary, are recognized as priority areas for biodiversity conservation and form an integral part of the proposed Nelson Mandela Metropolitan Open Space System (NM MOSS).

The NM MOSS prescribes a network of protected areas to ensure the conservation of a representative portion of biodiversity and natural features (SRK 2007a). The Swartkops River, Estuary and two Nature Reserves have been designated as Critical Biodiversity Areas (CBAs) that need protection. The Swartkops River/Estuary have been assigned a category of Critical Biodiversity Area 1 (CB1), with the recommendation that it should form part of the formal protected area system. The Swartkops Valley and Aloes Reserve complex have been assigned a category of Protected Area (PA1 or 2), with PA2 indicating that formal declaration is still pending (Aloes) (Enviro-Fish Africa 2011).

A vision for the Swartkops Estuary, the Swartkops Valley and Aloes Nature Reserve was set out as follows for the Swartkops Estuary Management Plan (EMP): “The Swartkops Estuary and the Swartkops Valley and Aloes nature reserves are unique national assets that are rich in biodiversity, and must be restored and protected to a level (Category B/C) that will attract visitors, uplift our spirits, sustain our livelihoods, and preserve our natural, cultural and recreational heritage” (Enviro-Fish Africa 2011).

In order to realise the vision of the Swartkops Estuary Management Plan, the health of the Swartkops Estuary needs to be improved in order to take it from the current category D health status to category C. This can be done by improving water quality, reducing fishing and bait collection in addition to the rehabilitation of the salt marsh and salt pans. Further actions that are required to improve the health of the Swartkops Estuary incudes restoring base flows and flooding events, rehabilitating riparian areas and wetlands, controlling recreational activities impacting on birds (van Niekerk et al. 2015), conducting a comprehensive Department of Water and Sanitation (DWS) ecological water requirement study to identify resource quality objectives (RQOs), requiring continuous long-term flow data to undertake a comprehensive assessment and thus installing a low-flow weir, installing a flow gauge closer to the head of the estuary to better quantify freshwater inflow, long-term monitoring of water quality to track improvement in addition to management of the catchment to reduce pollution into the estuary (Adams et al. 2019).

Taking the social and ecological importance of the Swartkops Estuary into account, justifies the use of this estuary to answer questions on the food resources available to the Cape stumpnose *R. holubi* in the seagrass habitat in the lower reaches of the estuary. The next chapter describes the geographical, biological and physical properties of the estuary.

Chapter 3: Study site description

The predominantly open Swartkops Estuary is located along the Eastern Cape of South Africa, 15 km north of Gqeberha Harbour at 33°51'54"S; 25°38'00"E. The estuary is ~16.4 km long and ~350 m wide in the lower reaches and narrows progressively towards the middle (~ 90 m wide) and upper reaches (Baird et al. 1986). The main catchment area lies in the Groot Winterhoek Mountains west of Uitenhage, covering an area of 1 303 km^2 and producing a mean annual runoff of ~84.2 x 10^6 m^3 (Reddering and Esterhuysen 1981; South African Environmental Observation Network (SAEON). The lower river receives water mainly from the Swartkops and Elands Rivers which flow parallel to one another before joining shortly after entering the floodplain (Baird et al. 1986).

Baird (2001) recorded a mean annual rainfall of ~636 mm, ranging from 500-1000 mm for the Swartkops Estuary. In 2021 and 2022 (data available from January to September), monthly total daily rainfall of 106.4 mm and 138.6 mm was greatest in July and August respectively (based on available data for this area from the South African Weather Service). An average daily maximum air temperature of 26.5 ºC was greatest in February for both 2021 and 2022 and an average daily minimum air temperature of 5.9 ºC and 7.9 ºC was lowest in July 2021 and June 2022 respectively (South African Weather Service).

Swartkops Estuary has a total estuarine habitat area of 926.7 ha (van Niekerk et al. 2019) and was ranked 11th out of more than 290 estuaries in South Africa in terms of its size, habitat diversity and biodiversity importance (Turpie et al. 2002). The estuary is categorised as 'largely modified' and has a Present Ecological State (PES) of Category 'D' (van Niekerk et al. 2015). The Present Ecological State defines the extent to which the present state differs from a natural condition that existed prior to human impacts (approximately 100 years ago). It ranges from natural (A) to critically modified (F) using an Estuarine Health Index (EHI). The estuary occurs in a densely urbanised area where surrounding residential and industrial anthropogenic activities (as follows) have significantly altered the water quality and health of the system (Adams et al. 2019).

Three residential nodes occur adjacent to the estuary, namely Bluewater Bay, Redhouse and Swartkops Village (Baird et al. 1986). The townships of KwaZakele and Motherwell are located further away from the estuary, but have a major effect on the system through pollution (Enviro-Fish Africa 2009). Industrial activities bordering the estuary include Fishwater Flats sewage treatment works, brickworks, sand/clay mining, abandoned saltpans, tanneries, motor vehicle and wool industries, marshalling railway roads, depots as well as extractive/beneficiation processes (Baird et al. 1986; Adams et al. 2019).

Overloaded and poorly managed sewage treatment plants, urban runoff and solid waste have all altered the water quality of the Swartkops River and Estuary. High nutrient loads and reduced river flows have favoured phytoplankton blooms (Lemley et al. 2015), which have resulted in eutrophic conditions in the middle and upper reaches of the estuary (Adams et al. 2019). Three wastewater treatment works (WWTWs) in the Swartkops catchment, namely Despatch, Kelvin Jones and KwaNobuhle are a major source of nutrients, with high levels of DIP (dissolved inorganic phosphorus) in the river attributed to discharges from these areas (Adams et al. 2019). This has promoted the growth of invasive aquatic plant species such as water hyacinth in the river. In addition to the three WWTWs, nutrient input from the Motherwell and Markman canals (draining township and industrial areas respectively) in addition to the Chatty River (draining highly populated township areas), are the main sources of pollution for the estuary. The Motherwell Canal is a significant source of nitrogen (in the form of ammonium) to the estuary (Adams et al. 2019). Faecal waste enters the middle reaches of the estuary via the Motherwell Canal due to leaks in the sewer system of the Motherwell Township area.

The Groendal Dam is the only major obstruction on the Swartkops River. It was constructed in 1939 and is located ~35 km upstream of the estuary (Baird et al. 1986). The dam has a storage capacity of ~12 x 10^6 m^3 and retains ~16 % of the mean annual runoff (MAR). Groendal Dam reduces freshwater inflow by ~5 % with the smaller Sand and Bulk River dams having little effect on freshwater. Below the Groendal Dam, five causeways span the Swartkops River and act as weirs that impede flow (Baird et al. 1986). In the area of Uitenhage and Despatch, four large road bridges and one railway bridge span the river. A retaining wall below the most westerly road bridge impedes flow and acts as a weir with the other bridges having little effect on flow (Baird et al. 1986). Even though the Groendal Dam has reduced freshwater inflow, discharge from the three upstream WWTWs (Despatch, Kelvin Jones and KwaNobuhle) have resulted in higher than natural flow conditions.

Swartkops Estuary comprises six different plant community types, namely benthic microalgae, phytoplankton, reeds, sedges, submerged macrophytes in addition to supra- and intertidal salt marsh (Colloty et al. 2002). The National Biodiversity Assessment (van Niekerk et al. 2019) reported the habitat area (ha) for open water (135 ha), intertidal salt marsh (209.2), supratidal salt marsh (338.15 ha), submerged macrophytes (44.7 ha) in addition to reeds and sedges (4.5 ha). Swartkops Estuary has the third largest salt marsh area in South Africa with intertidal salt marsh and intertidal benthic microalgae being the most important botanical components in the estuary based on a botanical importance rating system (Colloty et al. 2000; Adams et al. 2016). The salt marsh community has experienced the greatest loss in terms of habitat area due to residential and industrial development, most likely attributed to the reduction of tidal flow and lack of suitable engineering of these areas. Supratidal salt marsh was reduced in cover from 40 ha to 5 ha (88 % reduction) and intertidal salt marsh was reduced from 215 ha to 165 ha (27 % reduction), since before 1939 to 1996, as calculated from available maps, aerial photographs in addition to orthophoto maps (Colloty et al. 2000).

Zostera capensis occurs in the shallow waters of the Swartkops Estuary and is abundant in the channels between sand banks. This species is the dominant submerged macrophyte occurring in the estuary, with Swartkops having one of the largest areas of *Z. capensis* in South Africa after Knysna, Berg, Langebaan and Keurbooms estuaries (Adams 2016). The area cover of this species in the Swartkops Estuary has fluctuated over time (Table 3.1). The seagrass habitat is well known for its nursery function within estuaries, providing a source of food and refugia for juvenile fish and invertebrates (Beckley 1983; Edworthy and Strydom 2016).

Table 3.1: Changes in *Z. capensis* area cover in the Swartkops Estuary from 1939 to 2019

Year	**Area cover (ha)**	**Reference**
1939	24.77	Schmidt 2013
1957	15	Macnae 1957
1981	13.7	Talbot and Bate 1987 - Winter
1981	16.1	Talbot and Bate 1987- Summer
1982-1984	0	Emmerson et al. 1982 - flood led to complete removal
1996	12.5	Colloty et al. 2000
2013	44.7	Adams 2016
2019	62.3	Els et al. 2019

The macrobenthic species in the Swartkops Estuary are important prey items for fish and bird species and are vulnerable to exploitation by bait diggers as they inhabit accessible intertidal areas. The size of predatory bird populations and to a lesser degree those of fish, may be linked to the magnitude of prey populations, as observed for *Upogebia africana* (Underhill et al. 1980; Marais1984; Hanekom et al. 1988). Table 3.2 and 3.3 displays the global IUCN Red List Categories of some important fish and bird species, respectively, in the Swartkops Estuary.

For the purposes of this study, sampling sites were selected in the lower reaches of the Swartkops Estuary based on the presence of *Z. capensis* beds (Figure 3.1).

Figure 3.1: The location of the sites in the lower reaches of the Swartkops Estuary (labelled from 1 to 5) and the important areas surrounding the estuary

Table 3.2: IUCN Red List Categories of important fish species in the Swartkops Estuary

Species name	Common name	IUCN Red List Category
Argyrosomus hololepidotus	Madagascar Kob	Data Deficient (DD)
Atherina breviceps	Cape silverside	Not Evaluated (NE)
Diplodus sargus	White seabream	Least Concern (LC)
Gilchristella aestuaria	Estuarine round-herring	Least Concern (LC)
Lichia amia	Leervis	Least Concern (LC)
Lithognathus lithognathus	White steenbras	Endangered (EN)
Monodactylus falciformis	Full moony	Least Concern (LC)
Mugil cephalus	Flathead grey mullet	Least Concern (LC)
Mugil tricuspidens	Striped mullet	Not Evaluated (NE)
Pomadasys commersonnii	Smallspotted grunter	Least Concern (LC)
Rhabdosargus holubi	Cape stumpnose	Least Concern (LC)
Galeichthys feliceps	White barbel	Not Evaluated (NE)

(Baird et al. 1986)

Table 3.3: IUCN Red List Categories of important bird species occurring at Swartkops Estuary

Species name	Common name	IUCN Red List Category
Alcedo semitorquata	Half-collared kingfisher	Least Concern (LC)
Anas undulata	Yellow billed duck	Least Concern (LC)
Arenaria interpres	Ruddy Turnstone	Least Concern (LC)
Calidris alba	Sanderling	Least Concern (LC)
Calidris ferruginea	Curlew sandpiper	Near Threatened (NT)
Campethera notata	Knysna woodpecker	Near Threatened (NT)
Charadrius marginatus	White fronted plover	Least Concern (LC)
Charadrius pallidus	Chestnut-banded plover	Least Concern (LC)
Circus ranivorus	African marsh harrier	Least Concern (LC)
Egretta garzetta	Little egret	Least Concern (LC)
Falco biarmicus	Lanner falcon	Least Concern (LC)
Haematopus moquini	African black oystercatcher	Least Concern (LC)
Haliaeetus vocifer	African Fish Eagle	Least Concern (LC)
Hydroprogne caspia	Caspian tern	Least Concern (LC)
Larus cirrocephalus	Grey-headed Gull	Least Concern (LC)
Larus dominicanus	Kelp gull	Least Concern (LC)
Numenius phaeopus	Whimbrel	Least Concern (LC)
Pandion haliaetus	Western Osprey	Least Concern (LC)
Phalacrocorax capensis	Cape cormorant	Endangered (EN)
Phalacrocorax lucidus	White-breasted Cormorant	Least Concern (LC)
Phoeniconaias minor	Lesser Flamingo	Near Threatened (NT)
Phoenicopterus roseus	Greater flamingo	Least Concern (LC)
Platalea alba	African spoonbill	Least Concern (LC)
Pluvialis squatarola	Grey Plover	Least Concern (LC)
Podiceps nigricollis	Black-necked Grebe	Least Concern (LC)

Polemaetus bellicosus	Martial eagle	Endangered (EN)
Rostratula benghalensis	Greater painted snipe	Least Concern (LC)
Sterna balaenarum	Damara tern	Least Concern (LC)
Sterna dougallii	Roseate tern	Least Concern (LC)
Sterna hirundo	Common tern	Least Concern (LC)
Tadorna cana	South African Shelduck	Least Concern (LC)
Threskiornis aethiopicus	African sacred ibis	Least Concern (LC)
Xenus cinereus	Terek Sandpiper	Least Concern (LC)

(Baird et al. 1986; Martin and Baird 1987; Marnewick et al. 2015)

Chapter 4 provides an assessment of the epiphytic diatom assemblages on the leaves of *Z. capensis* in the lower reaches of the Swartkops Estuary, in order to identify whether this habitat provides for the prey resources most commonly found in the diet of *Rhabdosargus holubi* occupying this habitat.

Chapter 4: Diversity and abundance of epiphytic diatoms associated with *Zostera capensis* in the Swartkops Estuary

Introduction

Estuaries are shallow, sheltered environments that act as critical nursery areas for many species of marine fishes and invertebrates (Beck et al. 2001; Elliott and Hemingway 2008; Whitfield 2017). The nursery function of estuaries is attributed to the provision of structurally complex habitats (seagrass, salt marsh and mangroves) which increases the growth and survival of species by providing an abundance of food resources and protection from predators respectively (Heck et al. 2003; Schaffler et al. 2013, Whitfield 2020).

Many juvenile fishes have been observed utilising South African estuarine nurseries (Whitfield 2019). Of these, several are dependent on estuaries as exclusive nursery habitats and are either commercially or recreationally important fishes. One such species is the Cape stumpnose *Rhabdosargus holubi* (Sparidae) which is one of the most abundant marine fish species found in estuaries along the south-east coast (Whitfield 1998; Grant et al. 2017). This species migrates into estuaries as postflexion larvae and early juveniles during late winter, spring and early summer (Blaber 1974a; de Wet and Marais 1990). Environmental variables including temperature, turbidity and salinity have been observed to influence the recruitment dynamics of *R. holubi* into the Swartkops and Sundays estuaries (Kisten et al. 2015). *Rhabdosargus holubi* spends at least the first year of its life in estuaries before returning to the marine environment for maturation and spawning (Wallace et al. 1984). The higher temperature and rich food supply in estuaries, favours the rapid growth of this species (Blaber 1973; de Wet and Marais 1990).

The feeding habits of *R. holubi* differs as they grow, with the larvae feeding on zooplankton in the marine environment and the juveniles consuming mainly filamentous algae and aquatic macrophytes together with associated epiphytes and epifauna (de Wet and Marais 1990; Carassou et al. 2016; Nel et al. 2017). Adults in the marine environment are able to consume echinoderms, crustaceans, molluscs and polychaetes due to maturing juveniles undergoing dentition changes from the former sharp tricuspid incisors (in the outer row of both jaws) to molariform teeth (Buxton and Kok 1983; Carassou et al. 2016).

The feeding habits of *R. holubi* in the Swartkops Estuary examined from stomach contents of fish from the lower, middle and upper reaches of the estuary revealed that plant material, particularly *Zostera capensis*, dominated its diet, with crustaceans being the next most abundant, especially during winter (de Wet and Marais 1990). Although aquatic macrophytes are dominant in the diet of *R. holubi* juveniles, this species does not possess the enzyme cellulase to digest plant tissue (de Wet and Marais 1990). The plant material is, therefore, excreted in an undigested state, while the epiphytic algae and invertebrates occurring on the macrophytes are assimilated (Blaber 1974b; de Wet and Marais 1990). Results from isotope studies confirmed that epiphytic algae (especially diatoms) growing on aquatic plants are more important in the diet of *R. holubi* than the aquatic macrophytes themselves (Sheppard et al. 2012; Nel et al 2017). Diatoms are the dominant group of epiphytic communities occurring on aquatic macrophytes and they form an essential linkage in the food web structure of estuarine ecosystems, due to their nutritional value being greater than that of the macrophytes (Coleman and Burkholder 1995; Gordon et al. 2008). Epiphytes enter the food chain through selective feeding, accidental ingestion in addition to the breakdown of the host, thus entering the detrital pathway (Ziemann and Wetzel 1980).

Seagrass has been identified as an important nursery habitat for juvenile fish with most studies showing that the abundance, growth and survival of fishes were significantly greater in seagrass compared to unstructured habitats such as sand and mud flats (Hanekom and Baird 1984; Connolly 1994; Heck et al. 2003). Leslie et al. (2017) measured the habitat structural complexity of *Z. capensis* and *Spartina maritima* in the Bushmans Estuary according to six habitat complexity indices and related this complexity to the abundance and behaviour of *R. holubi*. Habitat structural complexity was found to be greater in *Z. capensis* than *S. maritima* according to four out of the six indices. The abundance of *R. holubi* was significantly greater in *Z. capensis* than in *S. maritima* and sand flats. Fish exhibited slow meandering behaviour in *Z. capensis*, which indicated a greater degree of habitat use than in the salt marsh and sand flat habitats. Foraging was also only observed in the seagrass habitat. The study by Leslie et al. (2017) showed that *R. holubi* in estuaries is associated with vegetation as well as prefer seagrass to salt marsh. Habitats of higher complexity provide a greater degree of protection from predators, and therefore, may also lead to increased survival (Bartholomew et al. 2000; Minello et al. 2003; Leslie et al. 2017). Nel et al. (2017) examined the feeding ecology of *R. holubi* across three vegetated sites (reeds, salt marsh and seagrass) in five temperate estuaries and found that overall, *Z. capensis* occurred most frequently in the stomachs of *R. holubi* from all habitats. This further reinforces the important role of seagrass in the ecology of this species.

Seagrasses provide a continuously renewing substratum for the colonisation of epiphytic organisms (Borowitzka et al. 2006). The life-span of seagrass blades, stems and rhizomes upon which epiphytes can grow, varies between seasons, habitats and species (Borowitzka et al. 2006). Seagrass is susceptible to die back over different seasons, which means that they differ in lengths at different times of the year, with higher biomass in summer and lower biomass in winter (Nienhuis and De Bree 1980). Seasonal patterns of certain epiphyte species have also been closely linked with the seasonality of their host seagrasses (Penhale 1977). Most epiphyte species peak in biomass in warmer months but the time of the peak varies between species (May et al. 1978). Some epiphyte communities peak in spring or summer and others peak in late summer and early autumn (May et al. 1978; Borowitzka et al. 1990; Edgar 1990).

This study aimed to identify the epiphytic diatom food resources available for juvenile fish, particularly for *R. holubi* in the seagrass habitat of the Swartkops Estuary. This habitat has been identified as a core nursery area for this species through a combination of greater abundance, higher growth (through food provision) and survival of juveniles (through protection from predation) (Leslie et al. 2017; James et al. 2019). Diatom assemblages were assessed between seagrass patches in the lower reaches of the Swartkops Estuary and the diversity and abundance of epiphytic diatoms scraped from the surface of seagrass leaves was determined during winter, spring and summer as *R. holubi* migrates into estuaries during late winter, spring and early summer. As the diet of *R. holubi* has been described (Blaber 1974b; de Wet and Marais 1990; Carassou et al. 2016; Nel et al. 2017; James et al. 2019), this study will compare what is found in the seagrass habitat and assess whether this habitat is suitable and useful for *R. holubi* in terms of known prey resources, addressing the following questions:

1) How do environmental variables differ seasonally in the *Z. capensis* beds and how does this influence the distribution of epiphytic diatom species?

2) What is the composition of the epiphytic diatom community available to *R. holubi* in the seagrass habitat of the Swartkops Estuary?

3) How does the epiphytic diatom community change temporally?

4) Is the seagrass habitat of the Swartkops Estuary a suitable nursery habitat for *R. holubi* in terms of food availability?

Materials and methods

Sampling procedure

Samples were collected in July (winter) and October (spring) 2019 and February (summer) 2020 during spring low tide from five sites in the lower reaches of the Swartkops Estuary (see "Chapter 3: Study site description: Figure 3.1" for further details) due to 90 % of the *Z. capensis* beds occurring within 6 km of the mouth of the estuary (Talbot and Bate 1987). Samples were not collected in autumn 2020 due to the COVID-19 pandemic. Nonetheless, autumn represents the period of slower primary producer growth and limited to no juvenile fish recruitment occurs into estuaries after summer (*sensu* Kisten et al. 2015). Therefore, the data collected are likely representative of key patterns related to the objectives of this study.

Physico-chemical variables

Three replicates each of water column salinity, temperature (°C), turbidity (FNU), dissolved oxygen (mg l^{-1}) and pH were measured at each of the five sites in the water column adjacent to the seagrass bed using a YSI ProDSS multiprobe during each sampling occasion. Physico-chemical variables were averaged for all five sites between seasons due to the close location of the sites in the lower reaches of the estuary (see "Chapter 3: Study site description: Figure 3.1" for further details), therefore, analyses were not performed between sites, only between seasons.

Sample collection

Seagrass with epiphytes were collected at each of the five sites based on the presence of *Z. capensis* beds at the time of sampling. Plant material lying within six 15 X 15 cm quadrats were removed by cutting the blades from the root base from each site. Three replicates were used to calculate epiphyte biomass and three replicates were used for epiphyte identification. Plant material was collected from the centre of seagrass beds and were placed into individually sealed plastic bags. Random blades were selected for processing.

Epiphyte chlorophyll *a*

Five seagrass blades, from each replicate collected to calculate epiphyte biomass from each site, were scraped on both sides with the back of a scalpel blade to remove the epiphytic layers (Dauby and Poulicek 1995). Distilled water was used to re-suspend the scraped material which was subsequently filtered through Whatman glass-fibre GF/C filters (Gordon et al. 2008) using a Millipore filter tower. The filters were placed into individual centrifuge tubes containing 30 mL of 90 % (v/v) acetone for 48 hours for the extraction of chlorophyll *a* (Gordon et al. 2008).

The samples were analysed using a 10-AU Turner Design fluorometer, fitted with narrow-band, non-acidification systems (Welschmeyer 1994). Each seagrass leaf blade was measured for leaf length and leaf width.

Chlorophyll *a* biomass was calculated using the following equation (Gordon et al. 2008):

$$\text{Chl } a = X \text{ x } \left(\frac{ml}{m^2}\right) \qquad \text{equ.1}$$

Where the units of Chl *a* is recorded as mg Chl *a* m^{-2}; X is the Fluorometer reading, *ml* is the volume of acetone and m^2 is the area of the blade scraped (calculated as leaf length multiplied by leaf width multiplied by two for both sides of the leaf).

Epiphyte species composition

Between three to five seagrass blades out of all replicates collected from each site for epiphyte identification were selected at random. These were scraped on both sides with the back of a scalpel blade to remove the epiphytic layers (Dauby and Poulicek 1995). The scraped material was preserved with 3 mL of glutaraldehyde and was stored in individual centrifuge tubes filled with 10 mL of distilled water. Diatom slides were prepared by digesting 10 mL of the scraped material in 20 mL of H_2O_2 (Taylor et al. 2007). Each sample was heated on a hot plate at 90°C for 1 to 3 hours. A few drops of HCl were added to each sample and it was left to cool. The samples were washed with distilled water by centrifuging five consecutive times at 2500 rpm for 10 minutes each. Two drops of the digested material were placed on a coverslip and left to dry with the coverslips being fixed to glass slides using pleurax mountant (available from Mr. Jonathan Taylor, North-West University). The dry slides were examined under a Zeiss Axioplan microscope at 1000X magnification. The relative abundance of the different species was determined by counting the first 200 diatom cells (or valves) per slide (Gordon et al. 2008). Diatoms were identified to the lowest possible taxonomic level where possible.

Data analysis

All statistical analyses were performed in R (v. 4.1.0) with statistical significance set a priori at $p < 0.05$. The Shapiro-Wilk test was used to test for normality and the Kruskal-Wallis test was used for non-parametric data, using the stats package in R (R Core Team 2021), to test for significant differences in physico-chemical variables and epiphyte chlorophyll *a* between seasons. Correlation analysis (Pearson) was performed between the environmental variables and epiphyte chlorophyll *a*, using the Hmisc package in R (Harrell Jr 2021).

Epiphyte abundance data was log (x + 1) transformed for the multivariate analyses to reduce the contribution of highly abundant species on the analysis. Analysis of similarity (ANOSIM) randomization tests were used to test for differences in species abundances between seasons and patterns were visualised using non-metric multidimensional scaling ordination (nMDS), using the vegan package in R (Oksanen et al. 2020). If differences were found using ANOSIM, then similarity of percentage (SIMPER) analysis was conducted to identify which species primarily accounted for observed differences in epiphyte assemblages between seasons, using the vegan package in R (Oksanen et al. 2020). SIMPER generates a ranking of the percent contribution of the species that are most important to the significant differences. These analyses used a matrix composed of Bray-Curtis similarity coefficients.

Using the vegan package in R (Oksanen et al. 2020), univariate indices including species richness (*S*) and diversity (Shannon-Wiener *H'*) were calculated for each season with a one-way ANOVA or Kruskal-Wallis test (depending on normality) used to test for overall differences between these indices between seasons, using the stats package in R (R Core Team 2021). The mvabund package in R (Wang et al. 2021) was used for a model-based analysis of the multivariate epiphytic diatom abundance data, with a anova.manyglm analysis performed to examine the effect of seasons on species abundances as in Warton (2008). Canonical correspondence analysis (CCA) ordination was used to investigate the relationship between epiphytic species and physico-chemical variables between seasons, using the vegan package in R (Oksanen et al. 2020).

Results

Physico-chemical variables

Table 4.1 indicates the physico-chemical variables during the study. Water temperature differed significantly between seasons (H = 43.15, df = 2, p < 0.001). It was significantly warmer in summer (21.57 ± 0.66 °C) than in spring (19 ± 0.78 °C) and winter (16.91 ± 0.40 °C). Salinity was significantly lower (H = 10.01, df = 2, p = 0.01) in winter (31.29 ± 2.32) than in spring (33.20 ± 0.30) and summer (33.27 ± 0.55). The pH was significantly (H = 23.5, df = 2, p < 0.001) higher in spring (8.33 ± 0.16) than in winter (7.95 ± 0.23) and summer (8.01 ± 0.15). Turbidity was significantly (H = 12.6, df = 2, p = 0.002) greater in winter (15.62 ± 11.30 FNU) and summer (10.90 ± 5.60 FNU) than in spring (9.83 ± 17.86 FNU). Dissolved oxygen (mg l^{-1}) did not differ between seasons (p > 0.05).

Table 4.1: Physico-chemical variables over all sampling periods (mean ± SD)

Sampling period	Physico-chemical variables				
	Temperature (°C)	Dissolved oxygen (mg l^{-1})	Salinity	pH	Turbidity (FNU)
Winter 2019	16.91 ± 0.40	9.44 ± 0.47	31.29 ± 2.32	7.95 ± 0.23	15.62 ± 11.30
Spring 2019	19.00 ± 0.78	9.03 ± 1.85	33.20 ± 0.30	8.33 ± 0.16	9.83 ± 17.86
Summer 2020	21.57 ± 0.66	8.24 ± 1.10	33.27 ± 0.55	8.01 ± 0.15	10.90 ± 5.60
H (Kruskal-Wallis test)	43.15	1.24	10.01	23.5	12.6
p value	<0.001	0.54	0.01	<0.001	0.002

Epiphyte chlorophyll *a*

Overall epiphyte biomass, calculated as chlorophyll *a* (mg m^{-2}) varied significantly between seasons ($H = 8.23$, $df = 2$, $p = 0.02$). It was significantly greater in winter (16.03 ± 12.57 mg Chl *a* m^{-2} blade area) than in spring (11.14 ± 8.37 mg Chl *a* m^{-2} blade area) (Figure 4.1).

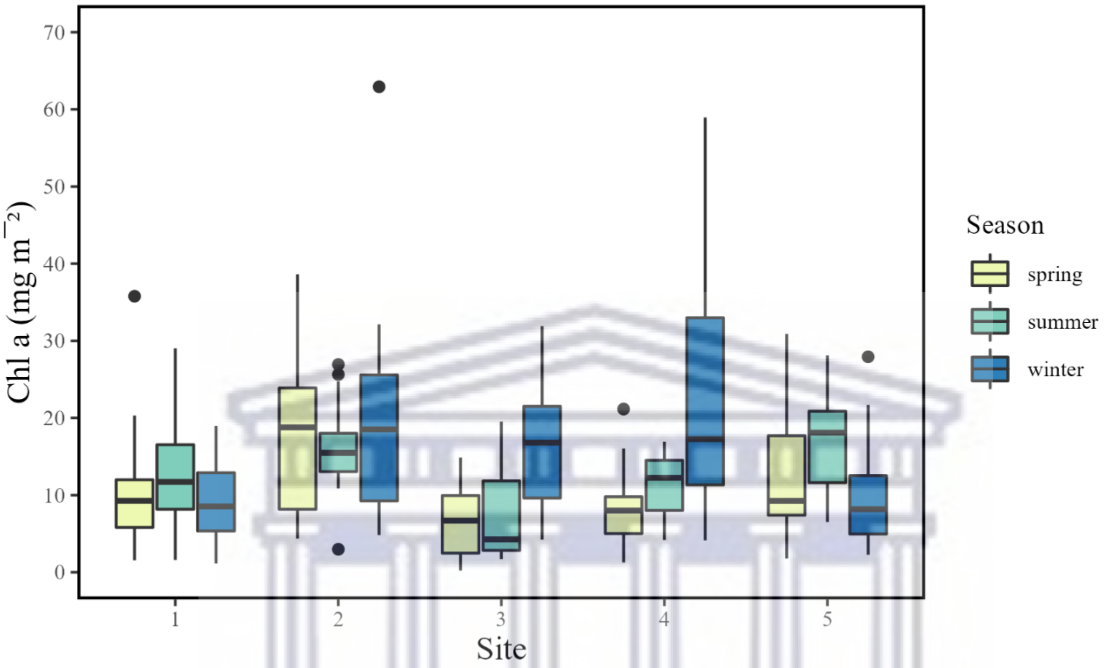

Figure 4.1: Epiphyte chlorophyll *a* concentration (mg m^{-2}) over all sampling periods. The box comprises three horizontal lines with the outer lines indicating the upper and lower quartiles of the data respectively. The length of the box indicates the interquartile range. The black horizontal line within the box indicates the sample median. The lines extending from the box represent the sample minimum and maximum. The circles lying outside of the interquartile range represent sample outliers

Epiphyte biomass did not display strong statistical relationships with environmental variables measured at each site as seen in the correlation matrix (Figure 4.2). The highest correlation of $r = -0.26$ was observed between epiphyte biomass (Chl *a*) and pH.

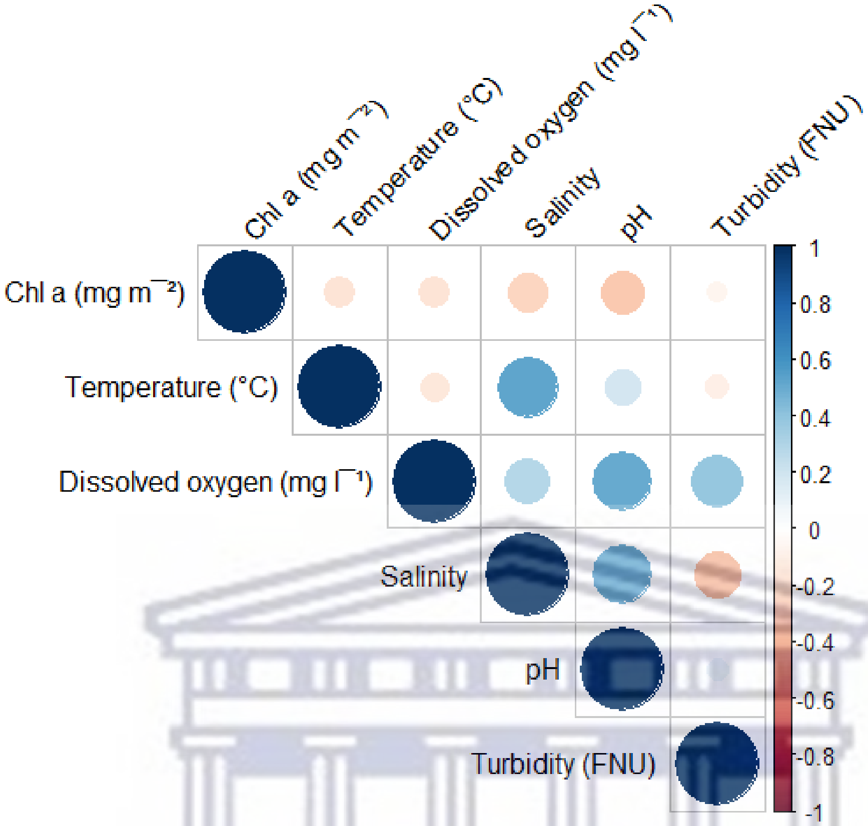

Figure 4.2: Pearson correlation matrix showing the relationship between epiphyte chlorophyll *a* (mg m^{-2}) and physico-chemical variables over all sampling periods. Positive correlations are displayed in blue and negative correlations are displayed in pink with colour and size being proportional to the correlation coefficient

Epiphytic diatom species composition

A total of 14 taxa of epiphytic diatoms were identified over all sampling periods (Table 4.2). Samples for epiphyte species composition were only collected from four out of the five sites for spring due to sparse diatom material on the leaves of *Z. capensis* from Site 3. The abundance of diatom species differed significantly between seasons (ANOSIM; $R = 0.6$, $p = 0.001$) (Figure 4.3). Analyses of similarity percentage (SIMPER) indicated that the seasons showing the greatest dissimilarity were winter and summer (57.93 %), followed by spring and summer (56.79 %), with winter and spring showing the lowest dissimilarity (43.68 %). Table 4.3 shows the average contribution of the most influential species to the overall dissimilarity observed between seasons. The taxon contributing most to the dissimilarities between winter and spring was *Tabularia* sp. 1, which was present in winter and spring but not summer. *Nitzschia angularis* was the species contributing most to the dissimilarities between winter and summer and spring and summer, this species was present during all seasons.

Table 4.2: "Presence-absence" species list of epiphytic diatoms identified on the blades of *Z. capensis* over all sampling periods (1 denotes present; 0 absent)

Species	Winter	Spring	Summer
Achnanthes sp. Bory, 1822	1	1	1
Cocconeis britannica Naegeli ex Kützing, 1849	1	1	1
Cocconeis engelbrechtii Cholnoky, 1955	0	1	1
Cocconeis sp. C.G. Ehrenberg, 1837	1	1	0
Grammatophora marina (Lyngbye) Kützing, 1844	1	1	1
Licmophora abbreviata C.Agardh, 1831	0	1	0
Mastogloia fimbriata (T.Brightwell) Grunow, 1863	1	1	1
Navicula sp. J.B.M. Bory de Saint-Vincent, 1822	1	1	1
Nitzschia angularis W.Smith, 1853	1	1	1
Nitzschia longissima (Brébisson) Ralfs, 1861	0	1	0
Nitzschia sp. A.H. Hassall, 1845	1	0	0
Nitzschia vidovichii (Grunow) Grunow 1881	1	1	1
Tabularia sp. 1 (F.T. Kutzing) D.M. Williams & F.E. Round, 1986	1	1	0
Tabularia sp. 2	1	1	1

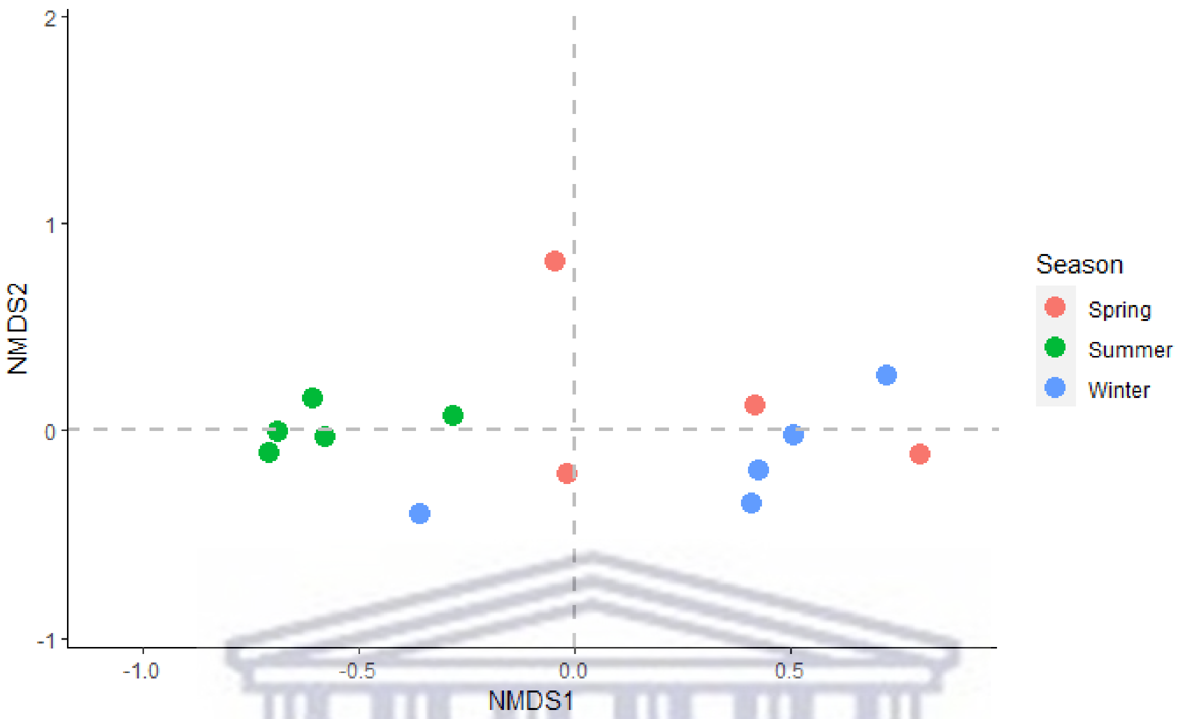

Figure 4.3: Non-metric multidimensional scaling (nMDS) ordination for the abundance of diatom species scraped from the blades of *Z. capensis* from sites in the lower reaches of the Swartkops Estuary. The abundance data was log (x +1) transformed and a resemblance matrix constructed using Bray-Curtis similarity (Stress = 0.09)

Table 4.3: SIMPER results showing the species contribution of the most influential diatom species to the average dissimilarity between seasons

Winter vs. Spring		Winter vs. Summer		Spring vs. Summer	
Species	Average Contribution (%)	Species	Average Contribution (%)	Species	Average Contribution (%)
Tabularia sp.1	5.42	*Nitzschia angularis*	10.57	*Nitzschia angularis*	9.75
Nitzschia longissima	5.07	*Tabularia* sp.1	8.94	*Mastogloia fimbriata*	7.36
Navicula sp.	4.54	*Mastogloia fimbriata*	7.27	*Nitzschia longissima*	6.09
Mastogloia fimbriata	4.34	*Navicula* sp.	7.26	*Nitzschia vidovichii*	6.05
Nitzschia angularis	3.73	*Cocconeis britannica*	4.47	*Tabularia* sp. 2	4.36
Tabularia sp.2	3.65	*Achnanthes* sp.	4.25	*Cocconeis britannica*	3.88
Nitzschia vidovichii	3.41			*Tabularia* sp. 1	3.83
Achnanthes sp.	3.35				

Although the abundance of diatom species differed significantly between seasons, the Shannon-Wiener diversity index (*H'*) showed no differences between seasons ($F_{(2,11)} = 2.97$, $p = 0.09$) (Figure 4.4A). Species richness (*S*), however, differed significantly between seasons ($F_{(2,11)} = 6.25$, $p = 0.02$) and it was significantly greater in spring (9 ± 2.16) compared to summer (5 ± 1.92) (Figure 4.4B).

Figure 4.5 shows the square root transformed abundance data for the diatom species observed in this study, with some species of epiphytic diatoms including *Mastogloia fimbriata*, *Cocconeis britannica* and *Nitzschia angularis* being more abundant and variable than others, such as *Licmophora abbreviata*, *Nitzschia* sp. and *Cocconeis* sp. Figure 4.6 shows the effect of seasons on individual species abundances per site, with only the most significant variables being shown based on univariate ANOVA *p*-values (Var = 73.16, $p = 0.002$).

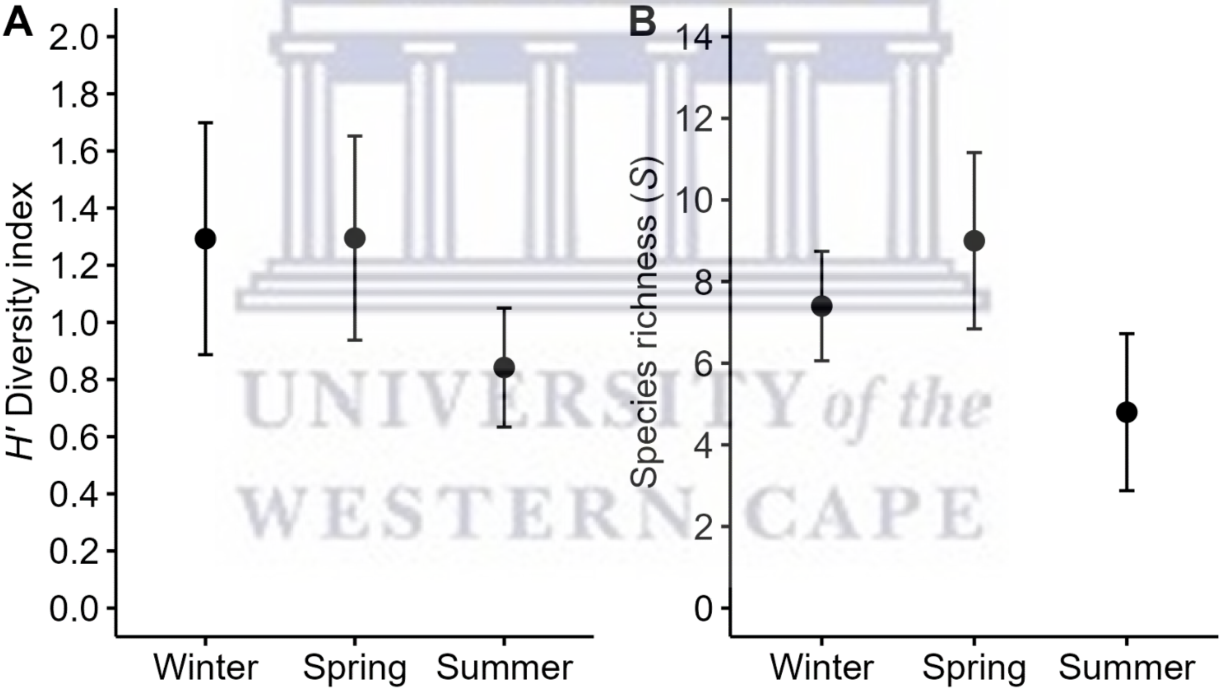

Figure 4.4: Mean Shannon-Wiener diversity index (*H'*) (A) and species richness (*S*) (B) for the abundance of epiphytic diatoms on the blades of *Z. capensis* over all sampling periods (Error bars showing standard deviation)

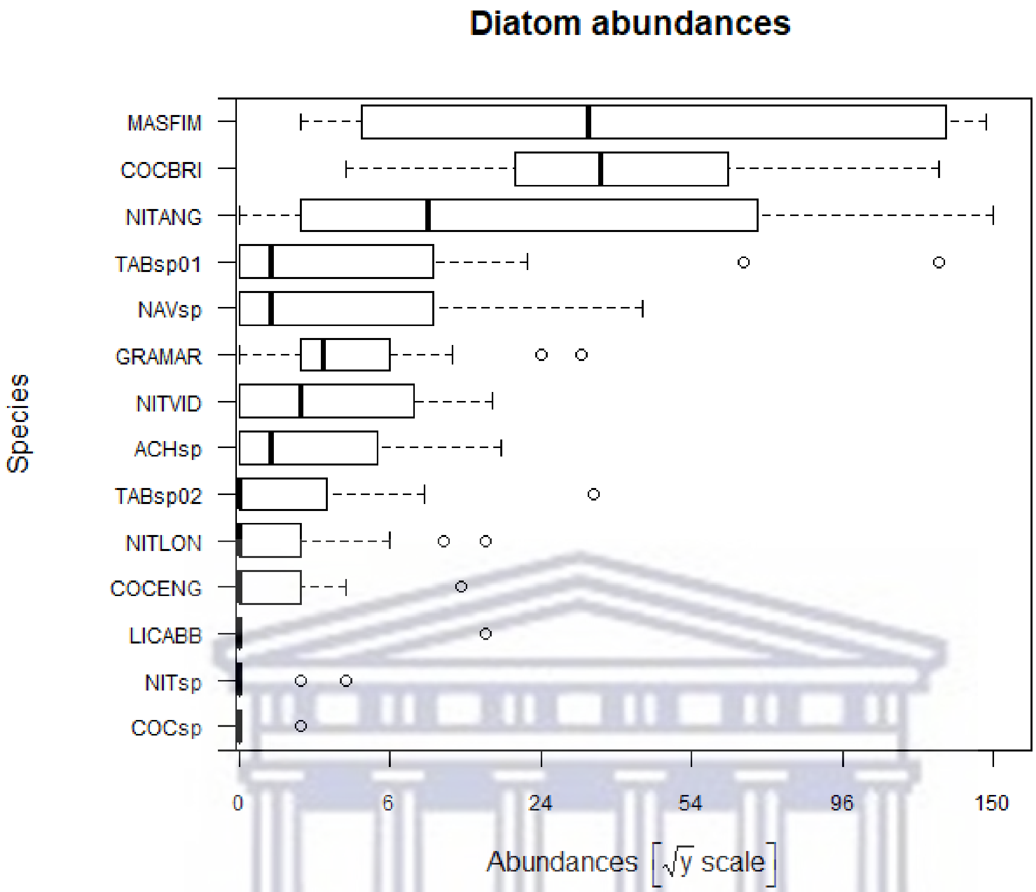

Figure 4.5: Boxplots showing the abundance of diatom species over all sampling seasons, produced using mvabund in R. Abundance data was square root transformed. ACHsp = *Achnanthes* sp., COCsp = *Cocconeis* sp., COCBRI = *Cocconeis britannica*, COCENG = *Cocconeis engelbrechtii*, GRAMAR = *Grammatophora marina*, LICABB = *Licmophora abbreviata*, MASFIM = *Mastogloia fimbriata*, NAVsp = *Navicula* sp., NITANG = *Nitzschia angularis*, NITLON = *Nitzschia longissima*, NITsp = *Nitzschia* sp., NITVID = *Nitzschia vidovichii*, TABsp01 = *Tabularia* sp. 1, TABsp02 = *Tabularia* sp. 2

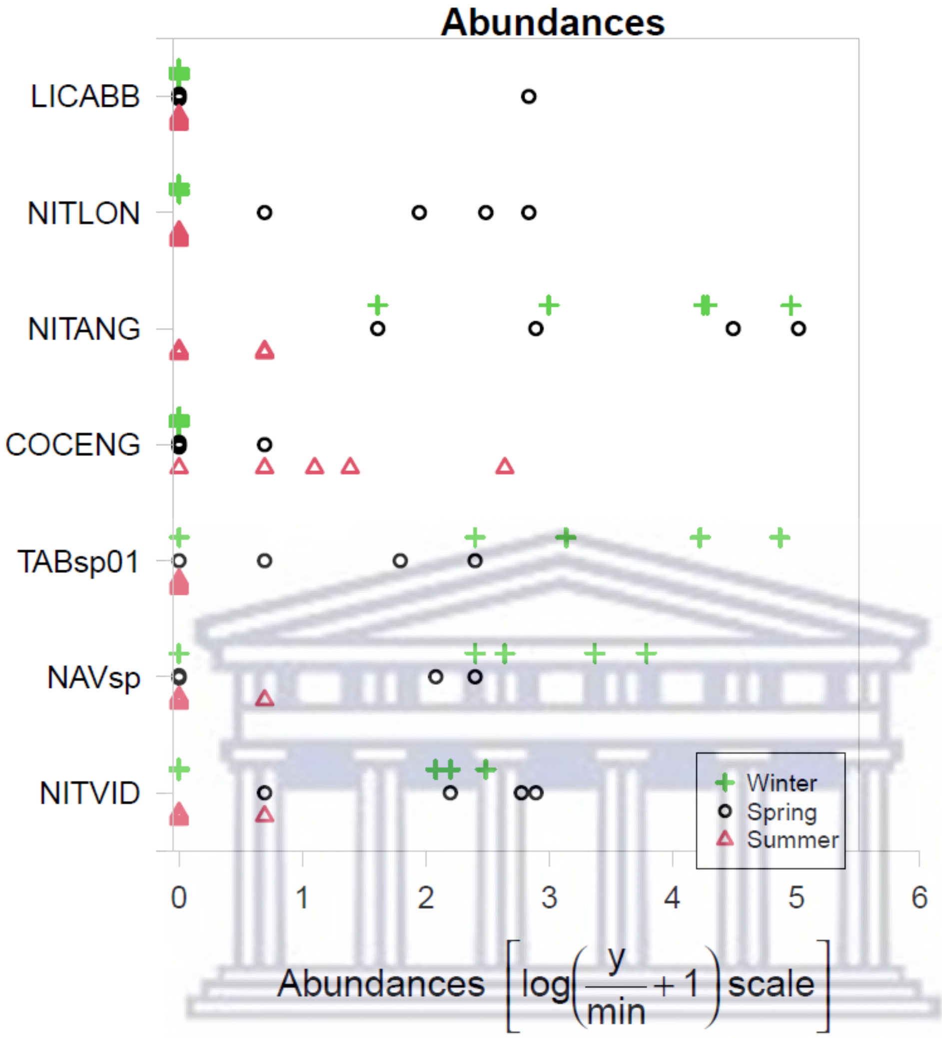

Figure 4.6: The abundance on a log (y +1) scale for diatom species per site which displayed the strongest evidence of a seasonal effect (as measured by exact permutation *p*-values based on ANOVA *F*-statistics for data transformed to log (y + 1). COCENG = *Cocconeis engelbrechtii*, LICABB = *Licmophora abbreviata*, NAVsp = *Navicula* sp., NITANG = *Nitzschia angularis*, NITLON = *Nitzschia longissima*, NITVID = *Nitzschia vidovichii*, TABsp01 = *Tabularia* sp. 1

Canonical correspondence analysis (CCA) indicated that axis I and axis II explained 69.03 % of the total variability in the abundance of epiphytic diatoms with respect to the environmental variables in this study. From the canonical correspondence analysis ordination (Figure 4.7), it can be observed that *Nitzschia* sp., *Cocconeis* sp. and *Mastogloia fimbriata* were found mostly at sites with high dissolved oxygen concentrations (mg l^{-1}) and low pH. *Tabularia* sp. 1, *Navicula* sp., *Nitzschia vidovichii* and *Nitzschia angularis* were found mostly at sites with high turbidity (FNU) and was most abundant during winter. *Achnanthes* sp., *Tabularia* sp. 2, *Nitzschia longissima* and *Licmophora abbreviata* were most abundant at sites with high pH and low dissolved oxygen concentrations (mg l^{-1}) and were most abundant during spring. It can also be seen that *Cocconeis engelbrechtii* and *Cocconeis britannica* were most abundant at sites with high water temperature (ºC) and salinity and during summer. *Grammatophora marina* was relatively close to the origin, meaning that the type of sites that this species was likely to be found at could not be described easily based on this ordination. It is possible that this species displayed no relationship with the physico-chemical variables or a quadratic relationship in which it was abundant at intermediate values of physico-chemical variables.

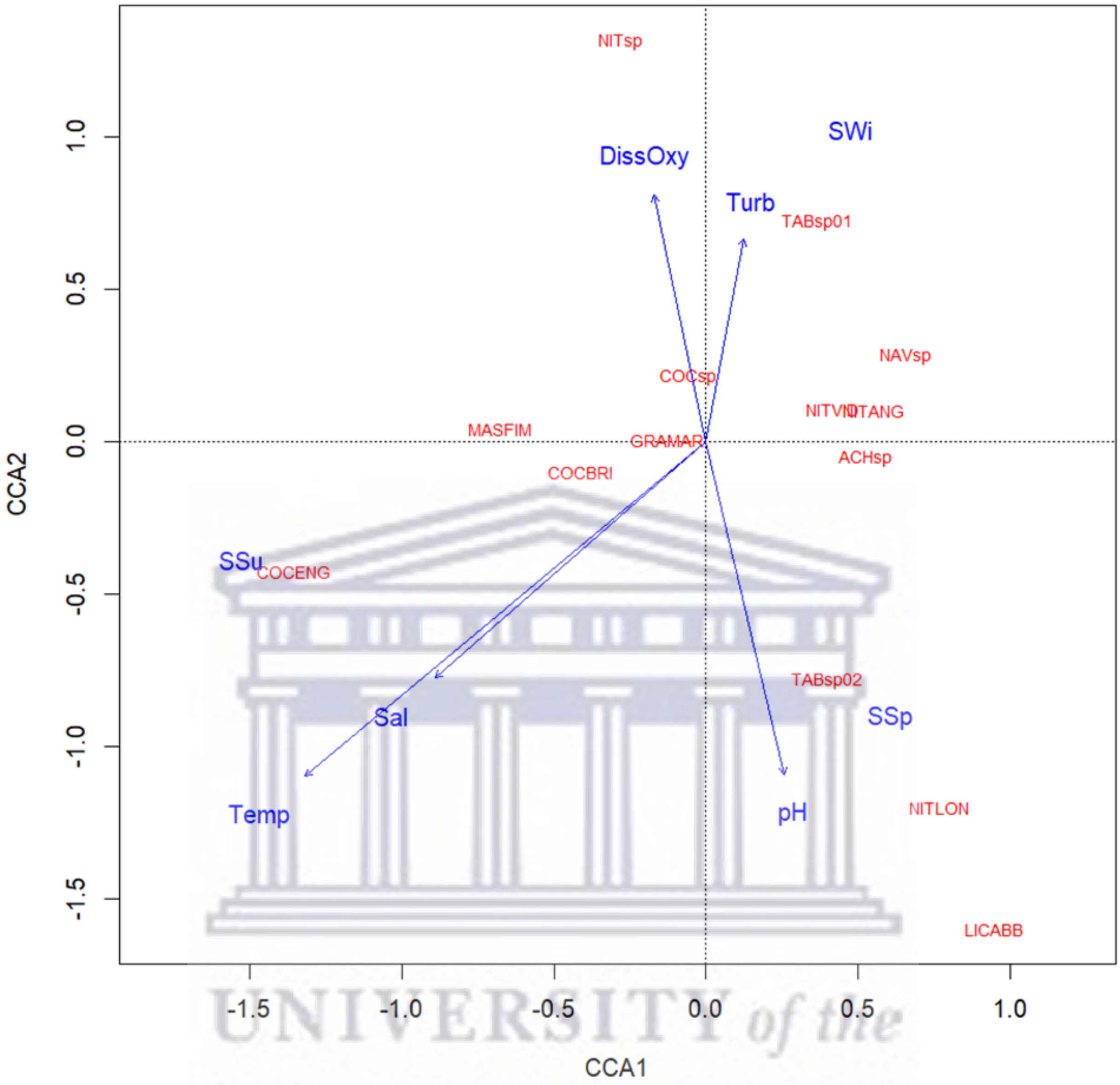

Figure 4.7: Canonical correspondence analysis (CCA) ordination of the log (x + 1) transformed abundance data of epiphytic diatom species against 5 physico-chemical variables over all sampling seasons. The length of the arrows represents how strongly each variable contributes to the ordination axes and the projections of each species to each physico-chemical variable axis indicate how strongly the abundances of that species are related to the physico-chemical variable. ACHsp = *Achnanthes* sp., COCsp = *Cocconeis* sp., COCBRI = *Cocconeis britannica*, COCENG = *Cocconeis engelbrechtii*, GRAMAR = *Grammatophora marina*, LICABB = *Licmophora abbreviata*, MASFIM = *Mastogloia fimbriata*, NAVsp = *Navicula* sp., NITANG = *Nitzschia angularis*, NITLON = *Nitzschia longissima*, NITsp = *Nitzschia* sp., NITVID = *Nitzschia vidovichii*, TABsp01 = *Tabularia* sp. 1, TABsp02 = *Tabularia* sp. 2, SWi = Winter,

SSp = Spring, SSu = Summer, DissOxy = dissolved oxygen (mg l^{-1}), Sal = salinity, Temp = temperature (ºC), Turb = turbidity (FNU)

Discussion

This study found temporal variation in the abundance of diatom species, with diversity showing no variation between seasons. Taxonomic richness was significantly greater in spring than summer. The abundance, diversity and distribution of epiphytic communities are affected by multiple abiotic and biotic factors, which occur at a variety of scales (Prado et al. 2007). The abiotic factors include light, temperature, water motion and nutrients (Mabrouk et al. 2011; Blake and Duffy 2016; Gauna et al. 2017) and the biotic factors include host leaf age, seasonal cycle of the host in addition to grazing pressure by herbivores (Gambi et al. 1992; Mazzella 1995; Prado et al. 2007; Mabrouk et al. 2011).

Water temperature in estuaries generally follow atmospheric conditions, with lows occurring during winter months and highs occurring during summer months (Russel and Montagna 2007; Vroom et al. 2017). Water column temperature in this study differed significantly between seasons, following a seasonal trend. It was warmer in summer and colder in winter and spring (Table 4.1). Salinity was significantly lower in winter than in spring and summer (Table 4.1), due to increased freshwater inflow during winter (Slinger and Taljaard 1994; Potter et al. 2016). Monthly total daily rainfall of 68.1 mm, was greatest in July 2019, when winter sampling for this study occurred (based on available data for this area from the South African Weather Service). The pH of the water column was significantly higher in spring than in winter and summer (Table 4.1), this is also most likely attributed to higher freshwater inflow during winter. The pH in estuaries is influenced by the pH in river inflow, which in turn is influenced by catchment characteristics such as the geology and vegetation cover (Bate et al. 2002b; Cai et al. 2013; Omarjee et al. 2021). The Swartkops catchment drains Table Mountain Group sandstone which typically has a lower alkalinity and electrical conductivity because of the slower dissolution rate, this lower ion content of the water results in a lower pH (Huizenga 2011; Nel et al. 2011; van Niekerk and Turpie 2012). The higher pH readings observed at the sites in the lower reaches of the estuary in this study, could have also been a result of higher marine intrusion occurring at the time of sampling (Dublin-Green 1990; Omarjee et al. 2020) The difference in turbidity could have been a result of human disturbance at these sites during field sampling. The oxygen concentrations recorded at the sites in the lower reaches of the estuary (Table 4.1) were indicative of a well-oxygenated system, with studies in warm temperate estuaries being well oxygenated with dissolved oxygen values mostly above 5 mg l^{-}

[1] (Emmerson 1985; Plumstead et al. 1985; Hecht and van den Lingen 1992; Scharler et al. 1997; Harrison 2004).

Overall epiphyte biomass was significantly greater in winter than in spring in this study and displayed weak statistical relationships with environmental variables. This suggests that biological variables such as grazing may have been important in determining epiphyte biomass distribution. In a study by Cattaneo (1983), experimental exclosures and enclosures showed that invertebrate biomass was the main factor influencing epiphyte biomass, rather than an unidentified climatic or seasonal factor influencing both epiphytes and grazers. In the study by Cattaneo (1983), peak biomass (between 23 and 25 mg Chl *a* m^{-2}) occurred during spring and autumn, while summer biomass levels were much lower (3 mg Chl *a* m^{-2}) as a result of invertebrate grazers causing the summer decline in epiphytes. In this study, the highest epiphyte biomass was observed in winter (16.03 mg Chl *a* m^{-2}), this may be indicative of decreased grazing occurring during this season. *Rhabdosargus holubi* displayed lower intensity feeding during winter in the Swartkops Estuary as observed by de Wet and Marais (1990), attributable to either lower water temperature or decreased food abundance or both. Schanz et al. (2002) found direct relationships between epiphytic biomass and grazing, with a thick layer of epiphytes growing on seagrasses (at high tidal current velocities) being caused by the low density of the grazer, *Hydrobia ulvae*, which in turn was a result of the hydrodynamic regime. The greater epiphyte biomass observed in winter in this study, may also be linked to a decrease in invertebrate grazers during this season. Prado et al. (2007) assessed the influence of biotic (meadow structure, herbivory and seagrass shoot length) and abiotic (light and nutrients) factors on integrative community measures (biomass, species richness and alpha-diversity) and species composition of epiphytes on the Mediterranean seagrass *Posidonia oceanica*. It was found that a large part of the variability in epiphyte species composition (51 %) was explained by the biotic and abiotic variables measured. Variability caused by differences in grazing pressure was the most important (25 %), followed by nutrient availability (11 %), meadow structure (6 %) , light (5 %) and seagrass shoot length (4 %). It was also found that among the integrative community measures, species richness was best explained by grazing and nutrients.

A total of 14 taxa of epiphytic diatoms were identified from the blades of *Z. capensis* in the lower reaches of the Swartkops Estuary in this study. These were not the only species that occurred on the seagrass blades as weakly silicified valves of other species could not be identified, and therefore, were excluded from this study. The most abundant diatom species observed in this study included *Cocconeis britannica*, *Mastogloia fimbriata* and *Nitzschia* *angularis*, which are marine diatom species, this is most likely due to the location of the sites being in the lower reaches of the estuary. It was observed that the epiphytic community on the leaves of the Mediterranean seagrass, *P. oceanica*, was dominated by species of the genus *Cocconeis*, most likely due to their high production rates, opportunistic colonisation strategy in addition to preferences for flat surfaces of seagrass leaves which comprises the bulk of the host plant body (Mazzella and Spinoccia 1992; Fourtanier and Kociolek 1999; Gacia et al. 2009). Other studies have also shown *Cocconeis* species of diatoms to dominate the epiphytic community of *Zostera* (Jacobs and Noten 1980; Chung and Lee 2008). In a study by Majewska et al. (2014), diatoms of the genus *Mastogloia* were also observed to dominate the epiphytic diatom communities colonizing *P. oceanica* along with seven other diatom genera, with individuals belonging to *Cocconeis* genus being the most abundant. *Nitzschia* is a widely distributed diatom genus comprising a large number of species, often being the most abundant taxa in inland, coastal as well as marine waters (Trobajo et al. 2004). *Nitzschia* spp. were observed to be dominant in the epiphyte diatom communities on the leaves of *Z. marina* and *Z. caespitosa*, distributed on the southern coast of Korea (Chung and Lee 2008).

The canonical correspondence analysis (CCA) in this study explained 69.03 % of the variability in the abundance of diatom species as a function of environmental variables and seasons, with temperature, pH and salinity being the most important environmental variables explaining the differences in the abundance of diatom species. Other factors including feeding by fish or birds, tidal action, river inflow and human disturbance may have also been important in explaining the variability in abundance, however, these were not measured in this study. Long-term studies have shown that seasonality in the abundance and diversity of seagrass epiphytes were observed to be a function of substrate availability (i.e., new seagrass surfaces), substrate stability (i.e., the life span of the seagrass leaf or stem which changes over the year), availability of larvae or propagules of epiphytes in addition to environmental conditions which favour the growth of epiphytes (Heijs 1984; Alcoverro et al. 1997; Reyes et al. 1998; Lepoint et al. 1999 and Wear et al. 1999). Grazing is another important factor that could have influenced the abundance of diatom species on seagrass leaves in this study.

Seagrass epiphytes are a food source for a wide range of grazers, who in turn, influence the abundance, diversity and distribution of epiphytic organisms (Borowitzka et al. 2006). Grazers control epiphytic algal biomass either directly through the removal of biomass or indirectly through the removal of the host substrate (Orth et al. 1984; Jernakoff et al. 1996; Borowitzka et al. 2006). The literature has shown that both vertebrate and invertebrate grazers can influence the abundance of epiphytic algae (Philippart 1995; Alcoverro et al. 1997; Fong et al. 2000; Heck et al. 2000), with estimates of up to 40 % of epiphytic algal production being lost to grazers (Peduzzi 1987). Grazers are also able to influence the structure of epiphytic assemblages through selective feeding (i.e., removing some species from an area and leaving the less preferred species) or the removal of competitive dominants (i.e., usually the faster growing species) which allows slower-growing, less competitive species to establish (van Montfrans et al. 1984; Jernakoff et al. 1996). Direct herbivory by fishes on seagrasses is rare, however, mugilids, hemiramphids, monacanthids, kyphosids and sparids have been reported to feed on epiphytic algae in seagrass beds (Jernakoff et al. 1996). This study aimed to identify the species of epiphytic diatoms available for the ubiquitous marine fish species, *R. holubi*, on the leaves of *Z. capensis* in the lower reaches of the Swartkops Estuary in addition to why the diatom species occur where they do in relation to environmental variables (CCA).

Multiple studies on the diet of *R. holubi* in estuaries have shown that filamentous algae, aquatic macrophytes and epiphytic algae were dominant components in the diet of this species (de Wet and Marais 1990; Carassou et al. 2016; Nel et al. 2017; James et al. 2019). It was previously hypothesized that macrophytes, although dominant in the diet of *R. holubi* juveniles, were poorly assimilated as this species does not possess the digestive enzyme cellulase (Blaber 1974b). Results from isotope studies have supported the above view that only the epiphytic algae (especially diatoms) covering the leaves of aquatic macrophytes are well digested and assimilated (Paterson and Whitfield 1997; Sheppard et al. 2012), and are therefore, more important in the diet than the macrophytes. Carassou et al. (2016), found that aquatic macrophytes were more prevalent in the diet of *R. holubi* from the lower reaches of the Kowie Estuary which is similar to what was found by de Wet and Marais (1990) in the Swartkops Estuary, with *Z. capensis* showing a great abundance in the stomachs of *R. holubi* collected from the lower reaches of the estuary. Possibly explained by the fact that more than 90 % of the *Z. capensis* beds occurred within 6 km of the mouth of the Swartkops Estuary (Talbot and Bate 1987).

James et al. (2019) compared the diatom composition on the leaves of *Z. capensis* to the diatom composition in the diet of *R. holubi* from different habitat types (seagrass, salt marsh, sand and mud flats), in the Bushmans Estuary, in order to confirm whether diatoms from seagrass beds constituted the main diet item for this species. Seagrass was the dominant component of the diet in all habitats during May (autumn) and it was only during this month that the diatom assemblage of fish caught in the seagrass habitat grouped together with the diatom assemblage on the seagrass leaves. This implies that individuals caught in the mud flats, sand flats and salt marsh habitats had been feeding in different seagrass patches as well as that diatom assemblages differ between different seagrass patches. The study by James et al. (2019), therefore, showed the importance of assessing differences in diatom assemblages between seagrass patches in order to understand important food resources available in different habitats, which was the aim of this study.

Results from the diatom analysis by James et al. (2019), revealed that *Grammatophora* species were the dominant diatoms in the diet of *R. holubi* in all seasons (except during winter) in the sand flats and seagrass habitats. *Grammatophora marina* was one of the most abundant diatom species identified on the leaves of *Z. capensis* in this study (Figure 4.5). Other species of diatoms that were identified in the diet of *R. holubi* by James et al. (2019) and occurred on the leaves of *Z. capensis* in this study, included *Cocconeis, Licmophora*, *Navicula* and *Nitzschia* species. As herbivorous fish species ingest aquatic plants and filamentous algae, they are likely to consume the diatomaceous layer occurring on the leaves of these aquatic plants and filamentous algae. The diatom composition in the diet, therefore, likely reflects the diatom assemblage occurring on plant material in the environment. Carassou et al. (2016) also showed that prey dominating the diet of *R. holubi* at different life stages, reflected food resource availability in the different habitats that were occupied by the fish at successive life stages.

Rhabdosargus holubi makes opportunistic use of different habitats according to food availability, shelter from predation and abiotic drivers including water depth and tidal phase. In the study by James et al. (2019), the salt marsh habitat was not an important feeding habitat for *R. holubi* as seagrass dominated the diet of fish captured in this habitat. This habitat ,however, did provide habitat complexity in the form of high plant biomass and canopy height (detailed in Leslie et al. 2017), which should provide *R. holubi* with protection from piscivorous fish predators during high tide. Leslie et al. (2017) found that slow meandering was the dominant behaviour of fish in the salt marsh habitat of the Bushmans Estuary during spring high tides, which indicates behaviour associated with a high degree of habitat use. Paterson and Whitfield (2000) found that fish (including *R. holubi*) caught in the salt marsh habitat of the Kariega Estuary at high tide, were also not using this habitat for feeding, suggesting that they were using it as a shelter from piscivorous fish predators. These examples show that salt marshes offer a retreat for *R. holubi* at spring high tides when large piscivorous fish have access to submerged seagrass beds and not to the shallower salt marsh habitats. James et al. (2019) showed the importance of invertebrate prey species in the stomachs of fish caught in the mud flats and sand flats that may not have been freely available in other habitats. The sand flats habitat, however, has a limited nursery value for *R. holubi* due to a low abundance of juvenile fish being observed in this habitat in addition to fish exhibiting rapid swimming behaviour (Leslie et al. 2017), suggesting that *R. holubi* juveniles pass through the sand flats and feed opportunistically in this habitat. The presence of seagrass in the stomachs of *R. holubi* caught in all habitats in the study by James et al. (2019), suggests that regardless of the habitat of capture, all fish fed on the epiphytes and epifauna associated with seagrass. James et al. (2019) also noted the contribution of red filamentous algae dominating the stomach contents of fish caught in the mud flats, sand flats and seagrass habitats, which was likely washed into the estuary from the sea. This suggests that *R. holubi* will feed opportunistically on red filamentous algal detritus that is transported up and down the estuary by tides, across all habitat types. Red algae are an abundant source of diatoms and can be consumed by *R. holubi* in greater quantities than seagrass when available as found by Blaber (1974b) in the Kowie Estuary.

Fish are capable of utilizing multiple habitats as seen above, which highlights the connectivity of shallow-water habitats and the broader concept of a seascape nursery. The seascape nursery, proposed by Nagelkerken et al. (2015), consists of connected mosaics of habitat patches instead of an individual entity, with hotspots of high animal abundances representing the core area of a seascape nursery. The seagrass habitat is a core nursey area for *R. holubi* attributed to food provision, shelter from predation in addition to supporting a great abundance of fish and invertebrates (Heck et al. 2003; Leslie et al. 2017; James et al. 2019). This study provided an assessment of the diatom assemblages within different seagrass patches in the lower reaches of the Swartkops Estuary in order to understand what important food resources (epiphytic diatoms) are available within different habitats.

Chapter 5 provides an assessment of the epifaunal and infaunal assemblages associated with seagrass beds in the lower reaches of the Swartkops Estuary in order to evaluate the invertebrate food resources available to *R. holubi*.

Chapter 5: Diversity and abundance of macrofauna associated on and beneath *Zostera capensis* in the Swartkops Estuary

Introduction

Many marine fishes depend on estuarine nursery habitats during their early life history stages in terms of food availability and protection from predators, which increases growth rates and survival respectively (Blaber and Blaber 1980; Beck et al. 2001; Heck et al. 2003). Submerged macrophyte beds within estuaries provide food-rich habitats that offer shelter from predation for many juvenile fishes, which further contributes to an increased ichthyofaunal richness, biomass and abundance (Hanekom and Baird 1984; Sheppard et al. 2012). The Cape stumpnose *Rhabdosargus holubi* (Sparidae) is a ubiquitous marine fish species that depends on estuarine nursery habitats during their early life stages (Blaber and Blaber 1980; Beck et al. 2001; Heck et al. 2003). This species migrates into estuaries during late winter, spring and early summer where it spends at least the first year of its life before returning to the marine environment for maturation and spawning (Blaber 1974a; de Wet and Marais 1990). Juveniles in estuaries consume mainly filamentous algae and aquatic macrophytes together with associated epiphytes and epifauna (de Wet and Marais 1990; Carassou et al. 2016; Nel et al. 2017). This species lacks the enzyme cellulase to digest plant material, which becomes excreted in an undigested state, while the epiphytic diatoms and invertebrates occurring on the macrophytes are assimilated (Blaber 1974b).

The contribution of invertebrates to the diet of *R. holubi* is essential from a nutritional perspective as this species is not effective at digesting plant material (James et al. 2019). Although epiphytic diatoms are assimilated by *R. holubi*, 73 % of diatom mass comprises indigestible siliceous frustules (Blaber 1974b). Sparids (including species such as gilthead seabream, European seabass and common dentex) require protein rich diet, estimated at 46–55 % protein according to Pavlidis and Mylonas (2011). Diatoms are rich in glycolipds, glycerides and poly-unsaturated fatty acids (Kates and Volcani 1966; Dunstan et al. 1996). A lipid content of between 9–17 % in the diet has been found to enhance protein retention in addition to increasing weight gain in sparids (Pavlidis and Mylonas 2011). A combination of protein and lipids is essential for sparid growth (Pavlidis and Mylonas 2011; James et al. 2019). Thus, it is the combination of protein from invertebrates and lipids from diatoms that is crucial for the growth of the sparid *R. holubi* in estuarine nursery habitats.

De Wet and Marais (1990) analysed the stomach contents of *R. holubi* in the Swartkops Estuary and determined an index of relative importance (IRI). *Zostera capensis* (Setchell 1933) was the most abundant item in their diet and together with filamentous algae, comprised the major proportion of the seasonal dietary pattern (IRI = 7314). This was followed by Crustacea (IRI = 6534) and Mollusca (IRI = 160). Crustaceans, however, were the principal component of the diet during winter. According to the IRI, crustaceans constituted an equally important portion of the seasonal dietary pattern, however, the mean mass per individual was virtually half that of plant material (de Wet and Marais 1990). Molluscs contributed a significant part of the diet with the pelecypods *Arcuatula capensis* and *Moerella tulipa* and the gastropod *Assiminea* spp., occurring most frequently. There was considerable variation in the quantities of all food items consumed by *R. holubi* in the Swartkops Estuary, with the composition always being the same. *Rhabdosargus holubi* vary their diet according to resource availability, which reduces competition for available food and allows alternative prey items to be consumed (de Wet and Marais 1990; Nel et al. 2017).

Using a combination of stomach contents and stable isotope methods, Carassou et al. (2016), showed that invertebrates dominated the diet of *R. holubi* in the Kowie Estuary. Juveniles consumed mainly invertebrates (amphipods, isopods and polychaetes) together with a mixture of aquatic macrophytes in addition to associated epiphyton and microphytobenthos. Nel et al. (2017) investigated the ecological feeding niche of *R. holubi* in different vegetated habitats (seagrass, salt marsh and reeds) across five temperate estuaries using stomach contents and stable isotope methods. Stomach contents revealed that Crustacea were the second most abundant food category following plant material. The amphipod, *Monocorophium acherusicum*, occurred most frequently in *R. holubi* guts occupying the reed habitat and in individuals greater than 80mm. *Hymenosoma orbiculare* and *Upogebia africana* were found in the stomachs of larger individuals occupying the salt marsh habitat. Bivalves were the third most abundant food category most likely due to their occurrence on the epiphytic algae found on the leaves of *Z. capensis* or from benthic feeding.

Prey dominating the diet of *R. holubi* reflects resource availability in the habitats that are occupied by this species at successive life stages (Carassou et al. 2016). As fish move between connected patches of habitat, they come into contact with different food resources. The interactions between estuarine fauna and their associated habitats are, therefore, important when evaluating the nursery value of a particular habitat type and for the implementation of conservation and management efforts (Hobson 1999; Adams and Paperno 2012).

This study identified the food resources available for juvenile fish, particularly for *R. holubi* in the seagrass habitat of the Swartkops Estuary. This habitat has been identified as a core nursery area for this species through a combination of greater abundance, higher growth (through food provision) and survival of juveniles (through protection from predation) (Leslie et al. 2017; James et al. 2019). This was be done by assessing the epifaunal and infaunal composition of seagrass beds in the lower reaches of the Swartkops Estuary from seagrass blades and the sediment respectively. The diet of *R. holubi* has been extensively described in the literature (Blaber 1974b; de Wet and Marais 1990; Carassou et al. 2016; Nel et al. 2017; James et al. 2019); this study compared what is found in the seagrass habitat and assessed whether this habitat provides for the prey resources most commonly observed in the diet of *R. holubi*. The epiphyton component within the seagrass habitat was outlined in Chapter 4. The present chapter therefore addresses the following questions:

1) What is the composition of the epifaunal and infaunal community associated with seagrass beds of the Swartkops Estuary?

2) How does this community change temporally?

3) Is the seagrass habitat of the Swartkops Estuary a suitable nursery habitat for *R. holubi* in terms of food availability compared to its known diet?

Materials and methods

Sampling procedure

Samples were collected in July (winter) and October (spring) 2019 and February (summer) 2020. Samples were collected from three sites in the lower reaches of the Swartkops Estuary, namely Site 1, 3 and 5 (see “Chapter 3: Study site description: Figure 3.1” for further details), based on the presence of seagrass beds. Samples were not collected in autumn 2020 due to the COVID-19 pandemic. Nonetheless, autumn represents the period of slower primary producer growth and no to limited juvenile fish recruitment occurs into estuaries after summer (*sensu* Kisten et al. 2015). Therefore, the data collected are likely representative of key patterns related to the objectives of this study.

Physico-chemical variables

Three replicates each of water column salinity, temperature (°C), turbidity (FNU), dissolved oxygen (mg l^{-1}) and pH were measured at each of the three sites in the water column adjacent to the seagrass bed using a YSI ProDSS multiprobe during each sampling occasion.

Epibiont and mobile epifaunal composition on and among the blades of *Z. capensis*

Three seagrass samples (the base the size of a R5 coin - sampling area of 5.31 cm^2) were removed from each site by cutting it at the water-sediment boundary with a scalpel (Whippo et al. 2018). Samples were placed into individual plastic jars filled with estuarine water and preserved with 30 ml of buffered formalin (4 %) and stained with Phloxin-B. Five random seagrass leaves were selected from each sample and the epibionts in the top 20 cm distal section of each blade were counted and the percentage cover recorded based on visual estimation (this included the presence of invertebrate eggs on seagrass blades). The mobile epifauna left in the container were also counted (occurring among the seagrass blades). Invertebrate macrofauna (> 0.5 mm) from the whole sample were identified to the lowest possible taxon where possible.

Infaunal composition in the sediment of seagrass beds

Infauna were sampled from the sediment in seagrass beds using a van Veen grab (sampling area 30 cm^2; depth 5 cm). Three replicate samples were collected at each site with each sample comprising three grabs (Pillay and Perissinotto 2008). Replicate sediment samples were emptied into a plastic bucket and estuarine water was added and the mixture was stirred vigorously in order to resuspend the benthic invertebrates. The supernatant was washed through a 500 µm sieve (Pillay and Perissinotto 2008). The process of adding water, stirring and sieving was repeated four times and any material that was retained on the sieve was emptied into a plastic jar. The remaining sediment was washed through a 2000 µm sieve in order to collect larger macrofauna including crustaceans, gastropods and bivalves (Cyrus and Martin 1988; Pillay and Perissinotto 2008). All macrofauna samples were preserved in 30 ml of buffered formalin (4 %) and stained with Phloxin-B. In the laboratory, macrofauna were counted and identified to the lowest possible taxon.

Data analysis

All statistical analyses were performed in R (v. 4.1.0) with statistical significance set a priori at $p < 0.05$. The Shapiro-Wilk test was used to test for normality and the Kruskal-Wallis test was used for non-parametric data, using the stats package in R (R Core Team 2021).

Macrofauna abundance (epibiont, mobile epifauna and infauna) data was log (x + 1) transformed for the multivariate analyses in order to reduce the contribution of highly abundant species on the analysis. Analysis of similarity (ANOSIM) randomization tests were used to test for differences in species abundances for all macrofauna between seasons and patterns were visualised using non-metric multidimensional scaling ordination (nMDS), using the vegan package in R (Oksanen et al. 2020). All observations (n=1) where no macrofauna were observed were removed when plotting the nMDS according to the assumptions and restrictions of Bray-Curtis similarity estimation. If differences were found using ANOSIM, then similarity of percentage (SIMPER) analysis was conducted, using the vegan package in R (Oksanen et al. 2020), to identify which species primarily accounted for observed differences in infaunal assemblages between seasons. SIMPER generates a ranking of the percent contribution of the species that are most important to the significant differences between seasons. These analyses used a matrix composed of Bray-Curtis similarity coefficients.

Using the vegan package in R (Oksanen et al. 2020), univariate indices including diversity (Shannon-Wiener *H'*) and species richness (*S*) were calculated for each season for all macrofauna with a one-way Kruskal-Wallis or ANOVA (depending on normality) used to test for overall differences between these indices between seasons, using the stats package in R (R Core Team 2021).

Canonical correspondence analysis (CCA) ordination was used to investigate the relationship between infauna and physico-chemical variables between seasons, using the vegan package in R (Oksanen et al. 2020).

The mvabund package in R (Wang et al. 2021) was used for a model-based analysis of the multivariate infaunal abundance data.

Results

Physico-chemical variables

Refer to “Chapter 4: Results: Physico-chemical variables”

Epibiont and mobile epifaunal composition on and among the blades of *Z. capensis*

A total of three epibiont and six mobile epifaunal taxa were identified on and amoung the blades of *Z. capensis*, respectively, between all sampling periods (Table 5.1). The abundance (n cm^{-2}) of epibiont taxa differed significantly between seasons (ANOSIM; $R = 0.62$, $p = 0.001$) (Figure 5.1) with the polychaete, *Spirorbis* sp., being significantly greater in summer than in winter and spring (Table 5.2) (invertebrate eggs were included in the epibiont analyses as it is a food resource for fish, which is what this study aims to quantify). The presence of invertebrate eggs (extrapolated as a per area estimate based on the number of blades measured compared to the number of blades in the sample) on the blades of *Z. capensis* was significantly greater in spring than in summer and winter ($H = 19.81$, $df = 2$, $p < 0.001$) (Table 5.2). The percentage cover of epibionts (based on the presence of epibiont taxa and invertebrate eggs) on the blades of *Z. capensis* was significantly greater in spring and summer than in winter ($H = 12.32$, $df = 2$, $p = 0.002$) (Table 5.2). The abundance of mobile epifaunal taxa (n cm^{-2}) did not differ between seasons (ANOSIM; $R = 0.06$, $p = 0.28$) (Figure 5.2; Table 5.3).

Table 5.1: “Presence-absence” species list of epibiont and mobile epifaunal taxa identified on and among the blades of *Z. capensis*, respectively, over all sampling periods (0 denotes absent; 1 denotes present; Unid. denotes unidentified species)

Class	Species	Winter	Spring	Summer
Epibionts				
Bivalvia	*Hiatula* sp. Modeer, 1793	1	0	0
Gastropoda	*Assiminea ovata* (Krauss, 1848)	1	1	1
Polychaeta	*Spirorbis* sp. Daudin, 1800	0	1	1
Mobile epifauna				
Gastropoda	*Assiminea ovata*	1	1	0
	Nassarius kraussianus (Dunker, 1846)	0	1	1
	Unid. mollusc 1	1	1	1
Malacostraca	*Hymenosoma orbiculare* Desmarest, 1823	0	0	1
	Palaemon peringueyi (Stebbing, 1915)	1	0	1
	Paridotea ungulata (Pallas, 1772)	0	1	0

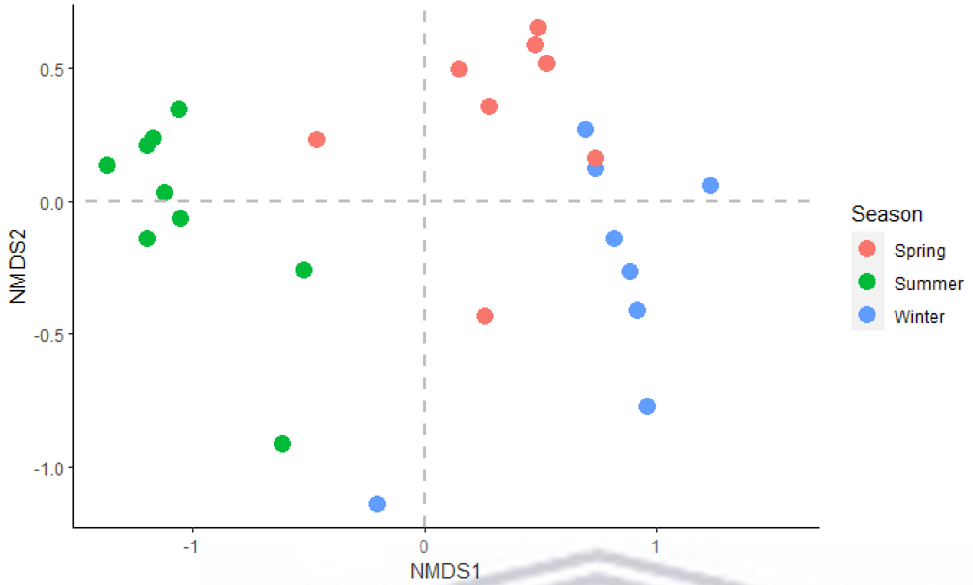

Figure 5.1: Non-metric multidimensional scaling (nMDS) ordination for the abundance (n cm^{-2}) of epibiont taxa identified on the blades of *Z. capensis*. The abundance data was log (x +1) transformed and a resemblance matrix constructed using Bray-Curtis similarity (Stress = 0.06)

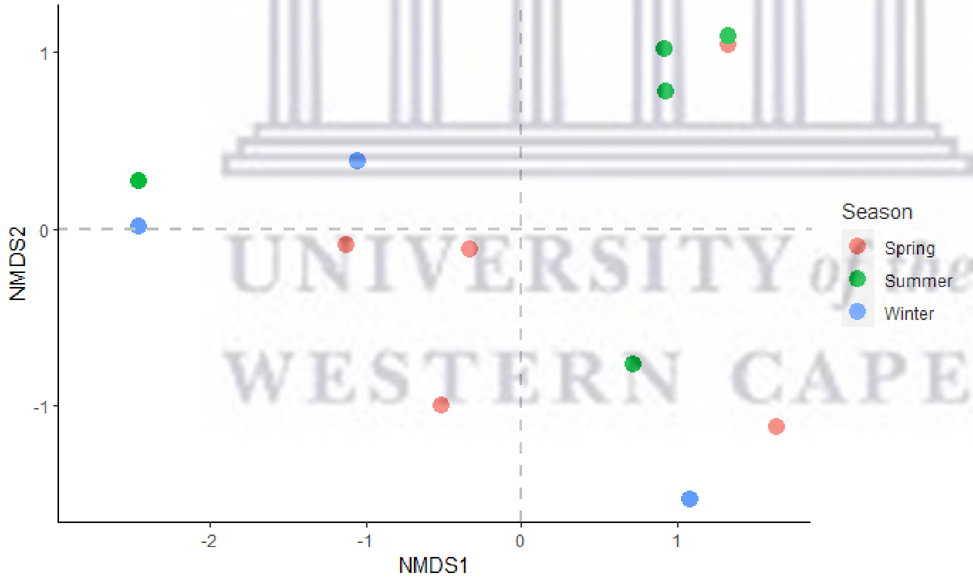

Figure 5.2: Non-metric multidimensional scaling (nMDS) ordination for the abundance (n cm^{-2}) of mobile epifaunal taxa identified among the blades of *Z. capensis*. The abundance data was log (x +1) transformed and a resemblance matrix constructed using Bray-Curtis similarity (Stress = 0)

Table 5.2: The mean abundance of epibiont taxa, number of blades, number of invertebrate eggs and percentage cover of epibionts associated with *Z. capensis* over all sampling periods

	Epibiont species (n cm^{-2})					
Season	*Assiminea ovata*	*Hiatula* sp.	*Spirorbis* sp.	Number of blades	Number of invertebrate eggs (n cm^{-2})	Percentage cover of epibionts
Winter	0.26	0.20	0	47.33	3.94	1.78
Spring	1.04	0	9.02	38.44	35.78	16.78
Summer	1.11	0	216.33	40	0.23	21.67

Table 5.3: The mean abundance of mobile epifaunal taxa and number of blades associated with *Z. capensis* over all sampling periods

	Mobile epifaunal species (n cm^{-2})						
Season	*Assiminea ovata*	*Hymenosoma orbiculare*	*Nassarius kraussianus*	*Palaemon peringueyi*	*Paridotea ungulata*	Unid. mollusc 1	Number of blades
Winter	1.15	0	0	0.82	0	2.01	47.33
Spring	1.53	0	0.69	0	1.69	0.63	38.44
Summer	0	1.15	2.26	0.94	0	1.15	40

The Shannon-Wiener diversity index (*H’*) and species richness (*S*) showed no differences between seasons for both the epibiont (*H’*: $H = 3.23$, $df = 2$, $p = 0.2$; *S*: $H = 5.02$, $df = 2$, $p = 0.08$) and epifaunal (*H’*: $H = 1.04$, $df = 2$, $p = 0.59$; *S*: $H = 1.47$, $df = 2$, $p = 0.48$) communities.

Infaunal composition in the sediment of seagrass beds

A total of 34 infaunal taxa were identified in the sediment of seagrass beds over all sampling periods (Table 5.4). The abundance of sediment infauna (n m^{-2}) differed significantly between seasons (ANOSIM; $R = 0.51$, $p = 0.004$) (Figure 5.3). Analyses of similarity percentage (SIMPER) indicated that the seasons showing the highest average dissimilarity were winter and spring (43.88 %), followed by winter and summer (40.92 %), with spring and summer showing the lowest dissimilarity (35.67 %). Table 5.5 shows the species contribution to the average dissimilarity between seasons.

Table 5.4: “Presence-absence” species list of infaunal taxa identified in the sediment of *Z. capensis* beds over all sampling periods (0 denotes absent; 1 denotes present; Unid. denotes unidentified species)

Class	Species	Winter	Spring	Summer
Bivalvia	*Hiatula* sp. Modeer, 1793	1	1	1
	Moerella tulipa (Hanley, 1844)	1	0	1
	Tellina sp. Linnaeus, 1758	1	1	1
Gastropoda	*Assiminea ovata* (Krauss, 1848)	0	1	1
	Nassarius sp. Duméril, 1805	1	1	1
	Nassarius capensis (Dunker, 1846)	1	0	1
	Nassarius kraussianus (Dunker, 1846)	1	1	1
	Natica sp. Scopoli, 1777	1	1	1
	Unid. mollusc 1	1	1	1
	Unid. mollusc 2	0	1	0
	Unid. mollusc 3	0	0	1
	Unid. mollusc 4	0	1	0
	Unid. mollusc 5	0	0	1
	Unid. mollusc 6	0	0	1
	Unid. mollusc 7	0	0	1
Malacostraca	*Hymenosoma orbiculare* Desmarest, 1823	0	1	1
	Palaemon peringueyi (Stebbing, 1915)	1	1	1
	Unid. decapod 1	0	0	1
Pilidiophora	*Gorgonorhynchus* sp. Dakin & Fordham, 1931	1	1	1
Polychaeta	*Glycera* sp. Lamarck, 1818	1	0	1
	Lagis pseudokoreni (Day, 1955)	1	1	1
	Magelona cincta Ehlers, 1908	1	0	0
	Owenia fusiformis Delle Chiaje, 1844	1	1	1
	Scolelepis sp. Blainville, 1828	1	1	1
	Scoletoma tetraura (Schmarda, 1861)	1	1	1
	Unid. polychaete 1	0	0	1
	Unid. polychaete 2	1	0	0
	Unid. polychaete 3	1	1	0
	Unid. polychaete 4	1	0	0
	Unid. polychaete 5	1	0	1
	Unid. polychaete 6	1	0	0
	Unid. polychaete 7	0	1	0
	Unid. polychaete 8	0	1	0
	Unid. polychaete 9	0	1	0

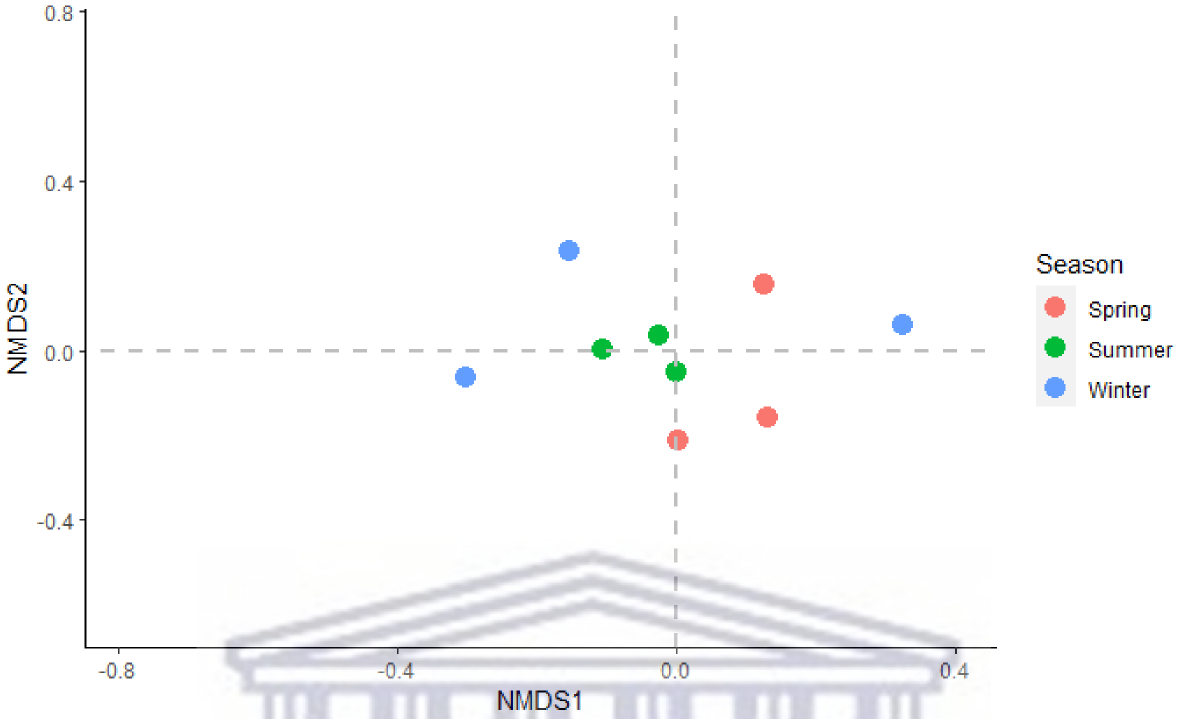

Figure 5.3: Non-metric multidimensional scaling (nMDS) ordination for the abundance (n m^{-2}) of infauna in the sediment of seagrass beds, collected from sites in the lower reaches of the Swartkops Estuary. The abundance data was log (x +1) transformed and a resemblance matrix constructed using Bray-Curtis similarity (Stress = 0.13)

Table 5.5: SIMPER results showing the species contribution of the most influential infaunal taxa to the average dissimilarity between seasons

Winter vs. Spring		Winter vs. Summer		Spring vs. Summer	
Species	Average Contribution (%)	Species	Average Contribution (%)	Species	Average Contribution (%)
Nassarius sp.	4.02	*Assiminea ovata*	3.97	Unid. mollusc 6	2.42
Glycera sp.	3.82	Unid. mollusc 6	2.53	*Assiminea ovata*	2.30
Tellina sp.	3.63	*Hiatula* sp.	2.42	*Hiatula* sp.	2.22
Assiminea ovata	3.03	Unid. decapod 1	2.33	Unid. decapod 1	2.22
Nassarius kraussianus	2.48	*Nassarius* sp.	2.15	*Nassarius kraussianus*	2.22
Nassarius capensis	2.39	*Glycera* sp.	2.09	*Nassarius* sp.	2.17
Lagis pseudokoreni	2.20	*Owenia fusiformis*	1.97	*Palaemon peringueyi*	1.88
Palaemon peringueyi	2.04	*Nassarius kraussianus*	1.89	*Tellina* sp.	1.84
Hymenosoma orbiculare	1.77	*Nassarius capensis*	1.72	*Hymenosoma orbiculare*	1.30
Owenia fusiformis	1.76	*Palaemon peringueyi*	1.68	*Natica* sp.	1.25
Unid. polychaete 3	1.66	Unid. polychaete 3	1.63	*Nassarius capensis*	1.24
Natica sp.	1.52	*Lagis pseudokoreni*	1.53	*Glycera* sp.	1.17
Unid. mollusc 1	1.51	Unid. mollusc 1	1.32	Unid. polychaete 1	1.17
		Scoletoma tetraura	1.29	*Gorgonorhynchus* sp.	1.08
		Scolelepis sp.	1.29	*Owenia fusiformis*	1.02

Although the abundance of infauna differed significantly between seasons, the Shannon-Wiener diversity index (*H'*) showed no differences between seasons ($H = 0.27$, $df = 2$, $p = 0.88$). Species richness (*S*) also showed no differences between seasons ($F_{(2,6)} = 1.99$, $p = 0.22$) (Figure 5.4).

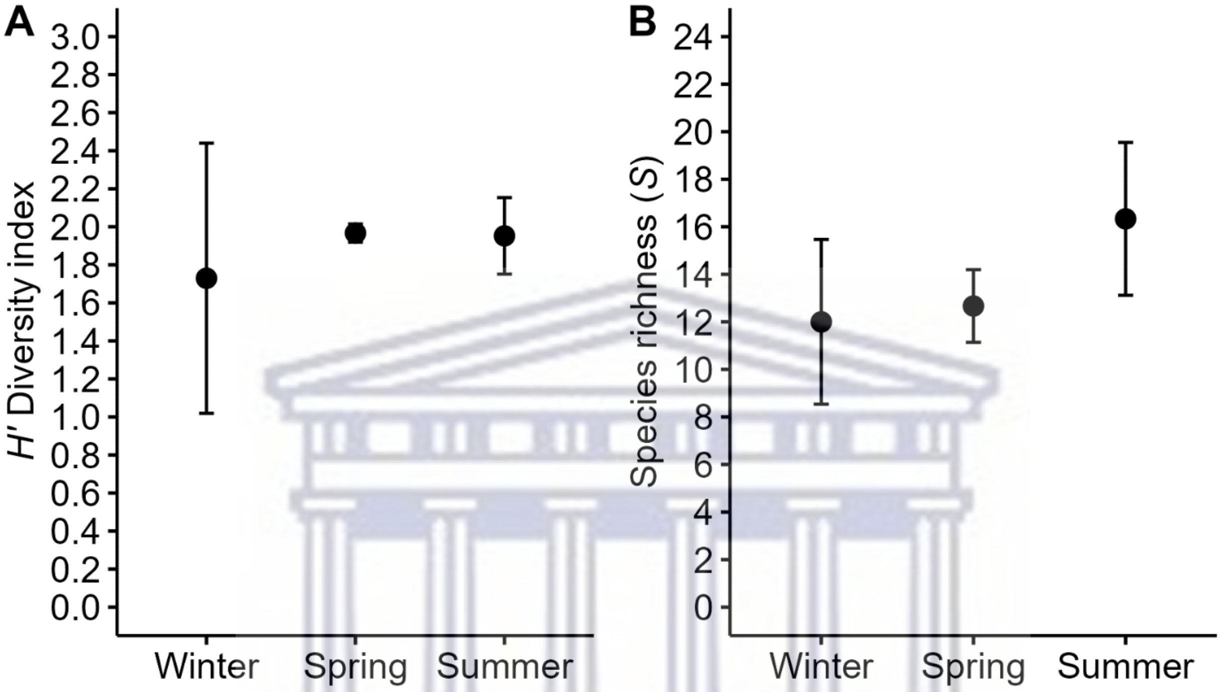

Figure 5.4: Mean Shannon-Wiener diversity index (*H'*) (A) and species richness (*S*) (B) for the abundance of sediment infauna over all sampling periods (Error bars showing standard deviation)

Canonical correspondence analysis (CCA) indicated that axis I and axis II expressed high cumulative variance, explaining 89.20 % of the total variability in the abundance of sediment infauna with respect to the environmental variables. From the canonical correspondence analysis ordination (Figure 5.5), it can be seen that unidentified mollusc 2, polychaete 7, polychaete 8 and mollusc 4 in addition to *Natica* sp., *Nassarius* sp., *Tellina* sp., *Hymenosoma orbiculare* and unidentified polychaete 9 were most abundant at sites with high pH and turbidity and during spring. *Lagis pseudokoreni* was also most abundant at sites with high pH. *Moerella tulipa*, *Palaemon peringueyi* and *Hiatula* sp. were most abundant at sites with low turbidity and pH. *Assiminea ovata* was most abundant at sites with high salinity and temperature. Unidentified polychaete 5, decapod 1, mollusc 7, polychaete 1, mollusc 6, mollusc 3 and mollusc 5 were also most abundant at sites with high temperature and during summer.

Unidentified polychaete 2, polychaete 4 and polychaete 6 in addition to *Magelona cincta*, *Nassarius capensis*, *Glycera* sp. and unidentified mollusc 1 were most abundant during winter and at sites with low temperature and salinity. Unidentified polychaete 3 was most abundant at sites with high dissolved oxygen. The remaining species including *Scolelepis* sp., *Scoletoma tetraura*, *Owenia fusiformis*, *Nassarius kraussianus* and *Gorgonorhynchus* sp. were relatively close to the origin, meaning that the type of sites these species were likely to be found at could not be described easily based on this ordination. It is possible that these species displayed no relationships with the physico-chemical variables or a quadratic relationship in which they were abundant at intermediate values of physico-chemical variables.

Figure 5.6 shows the square root transformed abundance data (n m^{-2}) of individual infaunal taxa over all sampling periods. *Scolelepis* sp. (76 m^{-2}), *Scoletoma tetraura* (76 m^{-2}), *Nassarius kraussianus* (54 m^{-2}) and *Owenia fusiformis* (53 m^{-2}) had the highest average abundance per m^{-2} over all sampling seasons. The abundance (n m^{-2}) of *Magelona cincta* ($F = 1$, $p = 0.002$), unidentified polychaete 2 ($F = 1$, $p = 0.002$), polychaete 4 ($F = 1$, $p = 0.002$), polychaete 6 ($F = 1$, $p = 0.002$) and *Glycera* sp. ($F = 9.06$, $p = 0.03$) was significantly greater in winter than in spring and summer. The abundance of unidentified mollusc 2 ($F = 1$, $p = 0.002$), mollusc 4 ($F = 1$, $p = 0.002$), polychaete 7 ($F = 1$, $p = 0.002$), polychaete 8 ($F = 1$, $p = 0.002$), polychaete 9 ($F = 1$, $p = 0.002$) and *Tellina* sp. ($F = 17.21$, $p = 0.01$) was significantly greater during spring than in winter and summer. The abundance of unidentified mollusc 5 ($F = 1$, $p = 0.002$) and *Hiatula* sp. ($F = 6.78$, $p = 0.03$) was significantly greater in summer than in winter and spring based on univariate ANOVA *p*-values.

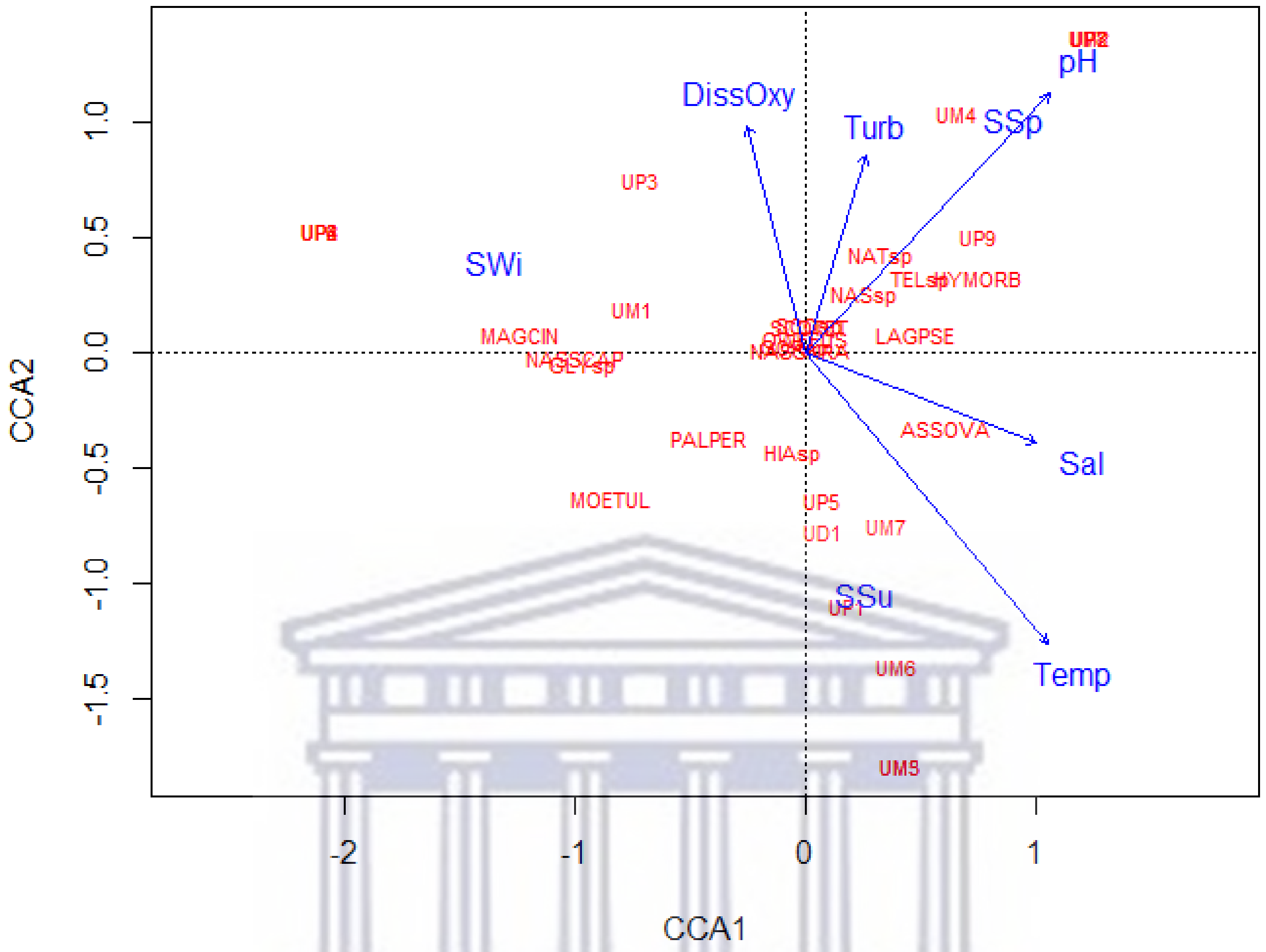

Figure 5.5: Canonical correspondence analysis (CCA) ordination of the log (x + 1) transformed abundance data of sediment infauna against five physico-chemical variables over all sampling seasons. The length of the arrows represents how strongly each variable contributes to the ordination axes and the projections of each species to each physico-chemical variable axis indicate how strongly the abundances of that species are related to the physico-chemical variable. ASSOVA = *Assiminea ovata*, GLYsp = *Glycera* sp., GORsp = *Gorgonorhynchus* sp., HIAsp = *Hiatula* sp., HYMORB = *Hymenosoma orbiculare*, LAGPSE = *Lagis pseudokoreni*, MAGCIN = *Magelona cincta*, MOETUL = *Moerella tulipa*, NASSCAP = *Nassarius capensis*, NASSKRA = *Nassarius kraussianus*, NASsp = *Nassarius* sp., NATsp = *Natica* sp., OWEFUS = *Owenia fusiformis*, PALPER = *Palaemon peringueyi*, SCOsp = *Scolelepis* sp., SCOTET = *Scoletoma tetraura*, TELsp = *Tellina* sp., UD = unidentified decapod, UM = unidentified mollusc, UP = unidentified polychaete, SSp = Spring; SSu = Summer, SWi = Winter, DissOxy = dissolved oxygen (mg l^{-1}), Sal = salinity, Temp = temperature (ºC), Turb = turbidity (FNU)

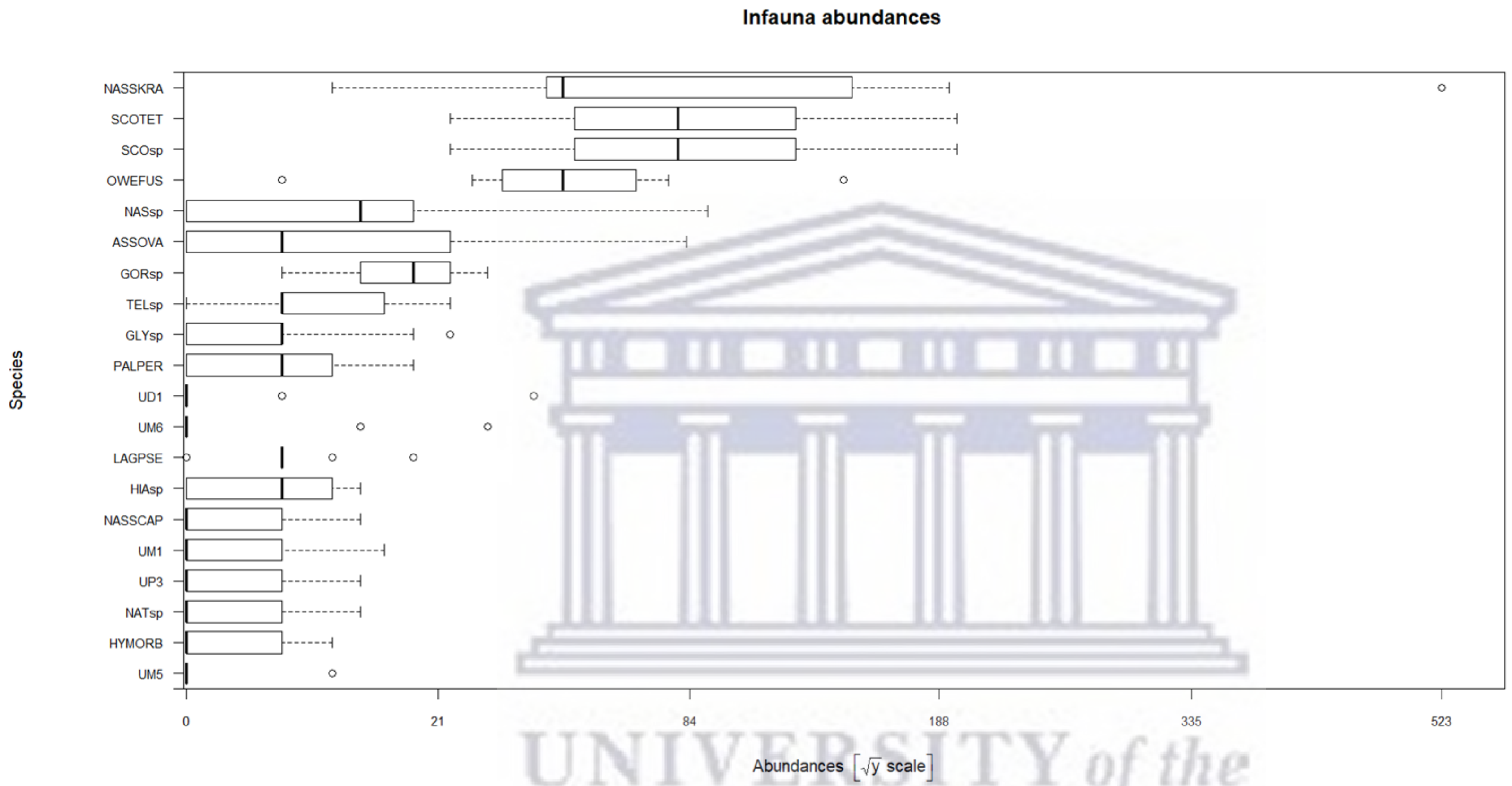

Figure 5.6: Boxplots showing square root transformed abundance data (n m^{-2}) of individual infaunal taxa (with only the 20 most abundant taxa shown) over all sampling periods. ASSOVA = *Assiminea ovata*, GLYsp = *Glycera* sp., GORsp = *Gorgonorhynchus* sp., HIAsp = *Hiatula* sp., HYMORB = *Hymenosoma orbiculare*, LAGPSE = *Lagis pseudokoreni*, NASSCAP = *Nassarius capensis*, NASSKRA = *Nassarius kraussianus*, NASsp = *Nassarius* sp., NATsp = *Natica* sp., OWEFUS = *Owenia fusiformis*, PALPER = *Palaemon peringueyi*, SCOsp = *Scolelepis* sp., SCOTET = *Scoletoma tetraura*, TELsp = *Tellina* sp., UD = unidentified decapod, UM = unidentified mollusc, UP = unidentified polychaete

Discussion

The abundance of epibiont and infaunal communities associated with *Z. capensis* beds in the lower reaches of the Swartkops Estuary displayed significant variation between seasons. The diversity and species number of these communities showed no significant variation between seasons. The mobile epifaunal community identified amoung the blades of *Z. capensis* also showed no variation in abundance, diversity and species number between seasons. Seagrasses are foundation species that support taxonomically and functionally diverse invertebrate assemblages comprising gastropods, amphipods, isopods and polychaete worms (Orth et al. 1984; Duffy et al. 2015). These species live on and among seagrass blades, connecting high epiphytic algal primary production to the production of larger invertebrates, fish and birds in the food chain (Hemminga and Duarte 2000; Duffy 2006). Understanding temporal and spatial variation is important in linking the abiotic and biotic characteristics of a marine seascape to the ecosystem functions that promote biodiversity and secondary productivity (Whippo et al. 2018).

The abundance, diversity and distribution of macrofaunal communities associated with seagrass meadows has been the focus of many studies (Ávila et al. 2015). These have found that faunal distribution can be influenced by both abiotic (e.g., dissolved oxygen, light, wind disturbance, hydrodynamics, sedimentation rate in addition to sediment particle size and composition) and biotic (e.g., predation, competition, food availability, leaf length, plant surface area in addition to seagrass density and biomass) factors (Lewis 1984; Livingston 1984; Boström and Bonsdorff 2000). Recent studies have focused more on regional-scale or multi-meadow processes such as dispersal (Whippo et al. 2018; Stark et al. 2020).

Whippo et al. (2018) quantified epifaunal biodiversity in a *Zostera marina* dominated seascape in Barkley Sound, British Columbia, in order to explore whether seagrass-associated biodiversity patterns were consistent with spatial processes such as abiotic habitat-filtering or metacommunity dynamics. Epifaunal community composition varied as much over a few meters within the same meadow as among meadows separated by kilometers and of different sizes and wave exposures. The spatial biodiversity patterns were not clearly explained by meadow location, area or abiotic conditions and may have been indicative of a metacommunity system. This suggests that processes maintaining diversity in eelgrass communities may reflect a seascape of many meadows connected through animal movement via dispersal. Stark et al. (2020) tested hypotheses about the contributions of species interactions, environmental filtering and dispersal to epifaunal invertebrate abundance and distribution associated with *Z.*

marina meadows, spanning an approximately 1000 km stretch of the British Columbia. Little support was found for complete dispersal limitation, with meadows in the same region often being no more similar to each other than meadows over 1000 km away. Environmental conditions such as temperature and dissolved oxygen explained a small amount of variation in taxonomic abundance patterns across the region and novel co-occurrence patterns were found among taxa that might have arisen from interspecific interactions. The results of the study suggested that seagrass-associated diversity patterns, reflect ecological processes spanning local (meadow) to regional scales.

In the study by Stark et al. (2020), the calcifying polychaete *Spirorbis* sp., dominated a seagrass meadow (HL) of the Haida Gwaii subregion. It was observed that this meadow had the lowest taxonomic richness (9 species) of all meadows and that there was fewer micro - and macroepiphytes on seagrass blades. The seagrass was completely covered with *Spirorbis* sp. This phenomenon has also been observed in *Z. marina* meadows in Akkeshi-ko Estuary, Japan by Smith et al. (2018) and by Dirnberger (1990) in *Thalassia testudinum* meadows in the northwestern Gulf of Mexico. The larvae of *Spirorbis* spp. tend to settle lower in the water column on newer seagrass growth, away from epiphytic algae and previously settled conspecifics, with settlement rates being determined by planktonic larval density instead of space availability on seagrass blades (Dirnberger 1990). It is possible that *Spirorbis* sp. dominated the HL meadow due to a combination of density-dependent processes such as high larval recruitment in addition to environmental variables such as high salinity and low nitrates. The low epiphyte load observed on seagrass at HL, whether mostly driven by high *Spirorbis* sp. densities or by the environment, may explain the low abundance and diversity of other invertebrate species at this meadow.

The abundance of *Spirorbis* sp. on the blades of *Z. capensis* in this study was significantly greater during summer than winter and spring. In chapter 4 of the results section of this dissertation, it was observed that the species richness (*S*) of the epiphytic taxa was lowest during summer (5 species). The fewer epiphytic species that were recorded on the blades of *Z. capensis* during this season, could be explained by the high density of *Spirorbis* sp. during summer. The epifaunal communities of *Zostera* may undergo large cyclical fluctuations in abundance with it usually peaking in spring to summer, with a corresponding decrease in winter (Marsh 1973; Marsh 1976). This could explain the higher percentage cover of epibionts observed on the blades of *Z. capensis* during spring and summer in this study in addition to the higher abundance of eggs observed on the blades in spring than in summer and winter.

Seasonality of reproduction and recruitment may also influence the temporal abundance of seagrass epifauna (Jernakoff et al. 1996; Whippo et al. 2018).

Macnae (1957) described the zonation of invertebrate species in the intertidal regions of the Swartkops Estuary. Similar mobile epifaunal species have been identified among *Z. capensis* blades in this study as have been collected in the “Zosteretum” by Macnae (1957) (22 species). These species include the crustaceans *Hymenosoma orbiculare* and *Paridotea ungulata* in addition to the molluscs *Assiminea ovata* and *Nassarius kraussianus*. Macnae (1957) also collected *Palaemon pacificus* in the Zosteretum with *Palaemon peringueyi* being identified in this study. *Hymenosoma orbiculare*, *Assiminea ovata*, *Nassarius kraussianus* and *Palaemon peringueyi* were also found in the sediment of seagrass beds in this study. In the study by Macnae (1957) most species displayed seasonal variation in numbers, with it being greatest in the winter months due to variation in temperature in the water of *Zostera* beds. During winter months, water temperature is relatively stable during the course of the day and may only rise a few degrees higher than the water in the channels of the river. During summer, however, the temperature range in *Zostera* pools fluctuates between highs and lows more considerably. Low water of spring tides usually occurs in mid-forenoon, which results in pools being exposed to the sun for some hours, with temperatures as high as 36 ºC having been recorded. Only the hardiest animals are able to survive in such warm water. Macnae (1957) observed *Nassarius kraussianus* to be the most abundant animal at any season, occurring in myriads. This species was also one of the most abundant infaunal species observed in the sediment of seagrass beds in this study. The shells of *Nassarius kraussianus* are always overgrown by the commensal hydroid, *Hydractinia kaffraria*, which was observed in this study.

A total of 34 infaunal taxa was observed in the sediment of seagrass beds in this study, which is much lower than the 160 species of invertebrate taxa that was observed in *Z. capensis* beds of the Knysna Estuarine Bay system by Barnes (2021). The Knysna Estuarine Bay is South Africa’s most significant system for biodiversity and conservation value. Of the 160 invertebrate species that were observed by Barnes (2021), *Assiminea ovata*, *Nassarius kraussianus* and *Hymenosoma orbiculare* were included in the more dominant members of the Knysna intertidal seagrass macrofauna. These species were also observed in the seagrass habitat in this study, with *Nassarius kraussianus* being one of the dominant infaunal taxa. Mclachlan and Grindley (1974) studied the distribution of macrofauna inhabiting the soft substrata of the Swartkops Estuary in relation to physical and biological factors. It was found that substrate and competition between communities were the most important factors limiting macrobenthic distribution. *Gorgonorhynchus* sp. was one of the few macrobenthic carnivores that were found in small numbers in the lower reaches of the Swartkops Estuary by Mclachlan and Grindley (1974), this species was also identified in this study. In the study by Mclachlan and Grindley (1974), *Palaemon pacificus* was one of the species to dominate the creek fauna of the Swartkops Estuary, with *Palaemon peringueyi* being identified in this study. Hanekom et al. (1988) recorded a total of 28 macrobenthic species in the soft substrata of the non-marshland areas of the Swartkops Estuary. Of the 28 species, only two were present in the sediment of seagrass beds in this study including *Hymenosoma orbiculare* and *Nassarius kraussianus*.

Recruitment and predation are major factors which influence the structure and function of marine infaunal communities between seasons. Recruitment is highly variable in space and time and is influenced by the hydrodynamical regime and food availability (Reiss and Kröncke 2005; Seitz 2011). Reiss and Kröncke (2005) studied seasonal variability in infaunal community structure in three areas in the North Sea under different environmental conditions. The seasonal variability in mean abundance, diversity and community structure of infauna was mainly due to recruitment in spring and summer. Thus, larval availability and settlement in addition to the consequent post-recruitment processes such as growth and mortality, all have a strong effect on the seasonal variability of benthic communities (Bosselmann 1991; Òlafsson et al. 1994). In a study by Sheridan and Livingston (1983) that sampled the infaunal community inhabiting a *Halodule wrightii* meadow in Apalachicola Bay, Florida, it was seen that the influx of juvenile fishes and crabs into the *Halodule* meadow during the summer months, led to a decline in infaunal population densities through predation. Predation was also observed to play a key role in controlling the density and community characteristics of infaunal communities in Upper Chesapeake Bay (USA), which varied with habitat type, season (i.e., predator abundance) and developmental or successional stage of the community as seen in the study by Holland et al. (1980).

James et al. (2019) found polychaetes, brachyurans, isopods, *Palaemon* shrimps, bivalves and gastropods in the stomachs of *R. holubi* in the Bushmans Estuary, with all of these taxonomic groups being well represented on the blades and in the sediment of *Z. capensis* in this study. Food items were expressed as a percentage of the total volume of stomach contents (%V) and the percentage frequency of occurrence (%F), which was the percent of stomachs that contained a specific food item. James et al. (2019) found that brachyurans 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 (V=2.5, F=1.8) and mud flats (V=2.0, F=6.7). Gastropods were found consistently (F>15%) in the diets of *R. holubi*, regardless of habitat. Based on frequency of occurrence (%F) and volume of consumed items (%V), Nel et al. (2017) found that *Hymenosoma orbiculare*, comprising < 2% of total volume, was generally found in the stomachs of *R. holubi* from *Spartina maritima* beds (F=14%) and larger fish individuals (81-140 mm) (F=23%). *Hymenosoma orbiculare* was also found among the blades and in the sediment of *Z. capensis* in this study. Carassou et al. (2016) also identified bivalves, brachyurans, gastropods and polychaetes in the stomachs of *R. holubi* in the Kowie system, with these taxonomic groups being well represented in this study. Based on frequency of occurrence (% F), Carassou et al. (2016) found that isopods, unidentified crustaceans and polychaetes (>10%) were the second most frequent dietary items in the diet of *R. holubi* juveniles from the river and estuary, which followed filamentous algae, amphipods, unidentified detrital material and aquatic macrophytes (>20%). As mentioned, de Wet and Marais (1990) found that molluscs contributed a substantial part of the diet of *R. holubi* in the Swartkops Estuary, with the pelecypods *Arcuatula capensis* and *Moerella tulipa* and the gastropod *Assiminea* spp. occurring most frequently. Of the gastropods, *Assiminea* spp. were the most abundant, occurring in low numbers and only in a few stomachs. Blaber (1974b) also reported *Assiminea* spp. to be present in the diet of *R. holubi* in both open and closed estuaries. *Moerella tulipa* was observed in the sediment of seagrass beds and *Assiminea ovata* was observed in both the sediment and on the blades of *Z. capensis* in this study.

Carassou et al. (2016) observed that the diet of *R. holubi* at different life stages reflected resource availability in the different habitats that were occupied by the fish at successive life stages. A study by James et al. (2019) that examined the diet of *R. holubi* juveniles in seagrass, salt marsh in addition to sand and mud flat habitats in the Bushmans Estuary, South Africa, showed that this species makes opportunistic use of different habitats according to food availability, shelter from predation as well as abiotic drivers such as water depth and tidal phase. The epifaunal and infaunal taxa identified in this study, therefore, likely reflects the resource availability for juvenile fish, particularly for *R. holubi* juveniles occupying the seagrass habitat of the Swartkops Estuary. Since *R. holubi* is not effective at digesting plant material, the contribution of invertebrates to the diet is critical from a nutritional perspective (James et al. 2019).

The settlement of epiphytic species, infauna, macrobenthic invertebrates and fish in seagrass meadows have resulted in these habitats having a higher production, abundance and diversity than unvegetated areas (Orth et al. 1984; Hemminga and Duarte 2000; Vonk et al. 2010). Despite the importance of seagrass meadows in supporting local and regional biodiversity, these ecosystems are experiencing a global decline due to human disturbances including coastal development and habitat destruction (as a result of boating and bait digging). This has resulted in the loss of *Z. capensis* area cover and biomass which has a cascading effect influencing higher trophic levels (Adams 2016). In South Africa, *Z. capensis* is extinct in the Durban Bay system due to habitat loss as a result of harbour development, reclamation and dredging and in the St Lucia system due to prolonged drought and closed mouth conditions (Adams and van der Colff 2018). *Zostera capensis* is now identified as endangered in South Africa (Adams and van der Colff 2018) with improved protection being required for this species, especially in estuaries with large important populations of *Z. capensis* such as Swartkops, Olifants, Berg, Keurbooms and Bushmans. Langebaan and Knysna estuaries do have some protection status (Adams 2016).

Chapter 6: General Discussion

The nursery-role hypothesis, defined by Beck et al. (2001), suggests that a combination of four factors including higher density, higher growth, survival of juveniles and movement corridors to adult populations, enhances recruitment of juvenile fish and invertebrates to adult populations. An abundance of energy rich food resources is essential for high growth rates and the estuarine habitats including seagrass, salt marsh and mangroves which provide this are, therefore, critical in facilitating the recruitment of juveniles to adult populations (Gibson 1994; Beck et al. 2001). Understanding the food resources used by fish species in estuaries, is important for quantifying the nursery value of different estuarine habitat types for juvenile fish. This study assessed the nursery value of the seagrass habitat in the lower reaches of the Swartkops Estuary for *Rhabdosargus holubi* in terms of epiphytic diatom and macrofauna food resource availability.

Rhabdosargus holubi makes use of estuarine habitats according to the availability of preferred food resources, macrophyte shelter from predation and in response to abiotic drivers such as water depth and tidal phase. Diet studies of *R. holubi* in estuaries revealed that this species feeds on the epiphytes (primarily diatoms) and epifauna associated with seagrass and red filamentous algae in addition to invertebrates (de Wet and Marais 1990; Sheppard et al. 2012; Carassou et al. 2016; Nel et al. 2017; James et al. 2019). Food items found in the diet of *R. holubi* were also found on the blades and in the sediment of *Zostera capensis* beds in this study, showing that this habitat does provide for *R. holubi* in terms of epiphytic diatom and macrofauna food resources. The temporal variation observed in the abundance of epiphytic diatoms and macrofauna in this study were most likely attributed to a number of interacting factors including environmental variables, substrate characteristics and grazing pressures. A high abundance of potential food resources is not necessarily a good indication of nursery potential, since a low abundance might show the same thing, only that high foraging may be occurring. This study shows that the seagrass habitat in the lower reaches of the Swartkops Estuary provides food resources for *R. holubi*, with the composition rather than the relative abundance of these indicating nursery suitability.

Epiphytic diatom and macrofauna food resources were available during all seasons in this study (with considerable variation in the abundance of individual taxa), showing seasonal consistency in resource availability. The presence of epiphytic diatoms and macrofauna in all seasons, shows that it is a reliable food resource for *R. holubi* as well as for other juvenile fishes. This study also suggested that grazing may have been important in determining the temporal variation observed in epiphyte biomass. Experimental exclosures and enclosures have shown that invertebrate biomass was the main factor influencing epiphyte biomass, with an increase in epiphyte biomass being linked to a decrease in invertebrate grazers and vice versa (Cattaneo 1983; Schanz et al. 2002). Epiphyte biomass was significantly greater in winter than in spring in this study (Chapter 4), which may be indicative of decreased grazing occurring during this season. In Chapter 5 of this study, it was observed that the polychaete *Spirorbis* sp. was significantly greater in summer than in winter and spring, with it being completely absent from seagrass blades during winter. The highest epiphyte biomass was observed in winter, which corresponds to the decrease in *Spirorbis* sp. on seagrass blades during this season. In Chapter 5, it was also observed that the percentage cover of epibionts on seagrass blades, was significantly greater in spring and summer than in winter. In Chapter 4, the species richness of epiphytic diatom taxa, was the lowest during summer (5 species), this could be explained by the high density of *Spirorbis* sp. during summer in addition to the greater percentage cover of epibionts on seagrass blades during this season.

The seagrass habitat has been identified as a core nursery area for *R. holubi* as it provides an abundance of food resources and protection from predators, which allows high specific growth rates and survival of juveniles respectively (Leslie et al. 2017; James et al. 2019). The significantly higher relative abundance of *R. holubi* observed in *Z. capensis* seagrass compared to *Spartina maritima* salt marsh and sand flats by Leslie et al. (2017) in the Bushmans Estuary, suggests that seagrass supports more *R. holubi* juvenile recruits to adult populations. This is due to relative abundance being related to density and studies using higher densities of juvenile fish as important indicators of emigration and recruitment (Minello 1999; Heck et al. 2003). Estuarine habitats may provide resources for juvenile fish without being considered a nursery habitat. This was observed in the sand flats habitat of the Bushmans Estuary by James et al. (2019), that provided invertebrate food resources for *R. holubi*, however, the low abundance of fish observed in this habitat, suggested that it was of limited value as a nursery for *R. holubi*. Refugia from predation likely diminishes the nursery potential of sand flats rather than food resource availability. The value of a critical estuarine nursery habitat depends on submerged aquatic vegetation providing optimum feeding and refuge opportunities, whilst also supporting a great diversity and abundance of fish and invertebrate species (Edworthy and Strydom 2016).

Seagrass ecosystems are experiencing a global decline due to direct (mechanical damage, eutrophication and coastal development) and indirect (negative impacts of climate change including erosion by sea-level rise and increased storms) anthropogenic disturbances, with the loss of these habitats leading to a decline in the abundance and diversity of juvenile fish that depend on these areas as nurseries (Duarte 2002). Sea-level rise is predicted to be a significant cause of seagrass decline (Hemminga and Duarte 2000). Erosion associated with sea-level rise and an increase in storm surges and high intensity rainfall events, will likely remove seagrass beds through uprooting (Duarte 2002). Sea-level rise will also affect the distribution of seagrass beds in estuaries due to changes in water depth and seawater intrusion (Short and Neckles 1999). Changes in the occurrence, spatial extent and functioning of estuarine macrophyte habitats in response to potential sea-level rise, therefore, needs to be documented in order to properly assess how sea-level rise will impact fish nursery areas (Whitfield 2017).

The evaluation of estuarine habitats as fish nursery areas has received significant attention in recent years, with the majority of studies suggesting that a habitat is a nursery due to supporting a higher density of juveniles relative to other habitats (Sogard and Able 1991; Rozas and Minello 1997; Bloomfield and Gillanders 2005). However, Beck et al. (2001), suggests that it is insufficient to use these single factors such as density, proof of feeding or protection from predators in isolation as proof of nursery provision and that multi-method approaches would provide better insight. Multi-method approaches quantifying growth, survival of juveniles and recruitment of sub-adults to adult populations are, however, rare due to the difficulty implementing them in a wide range of estuarine habitats. It is, therefore, necessary to develop more practical approaches to assess juvenile nursery habitats in estuaries. Future studies should focus on the nursery value of multiple habitat types in South African estuaries for *R. holubi* and other estuarine-dependent fish species. Studies should also focus on the nursery role of other submerged aquatic macrophyte species including *Potamogeton pectinatus* and *Ruppia cirrhosa*, occurring along the southern and eastern Cape coast of South Africa in addition to mangrove forests occurring along the east coast of South Africa northwards of the Nahoon Estuary, as little is known about the nursery value of these habitat types (Leslie 2016).

Estuaries are considered to be one of the most valuable aquatic ecosystems in the coastal zone attributed to their wide range of ecosystem services. Despite this, they are also one of the most degraded environments on earth due to being the focal points for human colonisation (Edgar et al. 2000). There is, therefore, widespread interest in the conservation and management of these coastal waters. Studies evaluating the nursery role of estuarine habitats, will provide insight on the nursery value of different habitat types, which will inform ecosystem management and conservation plans (Beck et al. 2001). The link between threatened estuarine habitats such as seagrasses and the communities that depend on them, also highlights the need for an ecosystem-based management approach that incorporates interdependencies and facilitation between species (Hughes et al. 2009), which is essential for effective conservation.

An assessment of the epiphytic diatoms and macrofauna associated with *Z. capensis* beds in the middle reaches of the Swartkops Estuary in this study, would have been useful in identifying additional taxa that were not represented in the lower reaches. The composition of the epiphytic diatom and macrofauna communities were similar across all sites based on their location in the lower reaches of the estuary, so a broader study area (not confined to the lower reaches) would have incorporated a wider range of epiphytic diatom and macrofauna taxa. To further improve this study, an assessment of food availability during autumn would have been beneficial in a comparison of the temporal variation in the abundance and diversity of epiphytic diatom and macrofauna species, however, this was not possible due to the COVID-19 pandemic. Future studies should expand on the diatom assemblages associated with filamentous algae, which is abundant in the lower reaches of the Swartkops Estuary, as de Wet and Marais (1990), found that *Z. capensis* together with filamentous algae, comprised the major proportion of the seasonal dietary pattern of *R. holubi* juveniles in the Swartkops Estuary. Future studies should also assess the nursery value of the seagrass habitat for other estuarine-associated fishes including Cape silverside *Atherina breviceps,* estuarine round-herring *Gilchristella aestuaria* in addition to white seabream *Diplodus sargus*, that dominate this habitat in the Swartkops Estuary.

This study showed the importance of *Z. capensis* seagrass beds in providing an abundance of food resources for juvenile fish, with the loss of this habitat, further influencing the communities that depend on them. Human disturbances such as boating and bait digging have reduced *Z. capensis* area cover and biomass in South African estuaries. Boating can reduce seagrass cover through physical removal by propellers in addition to bank erosion and by increasing turbidity (Adams 2016). Trampling as a result of bait digging and pumping for mud and sandprawns, have caused localised extinction of *Z. capensis* in Langebaan Lagoon (Pillay et al. 2010). Trampling, sand excavation and scouring due to bivalve collection in addition to fishing gear, has also significantly reduced the extent of *Z. capensis* beds in Maputo Bay (Bandeira and Gell 2003). Swartkops Estuary has one of the largest subpopulations of *Z. capensis* in South Africa and, therefore, it is essential that this habitat is properly managed due to the important resources that it provides for juvenile marine fish, which is integral for their survival and recruitment to adult populations.

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Appendix

Appendix Table 1: Abundance (n cm^{-2}) of epibiont taxa per site per season

		Species Abundance (n cm^{-2})		
Season	Site	*Hiatula* sp.	*Assiminea ovata*	*Spirorbis* sp.
Winter	1	0	0	0
Winter	1	0	2	0
Winter	1	0	0	0
Winter	3	0	0	0
Winter	3	0	0	0
Winter	3	0	0	0
Winter	5	2	0	0
Winter	5	0	0	0
Winter	5	0	0	0
Spring	1	0	0	81
Spring	1	0	3	0
Spring	1	0	0	0
Spring	3	0	0	0
Spring	3	0	1	0
Spring	3	0	5	0
Spring	5	0	0	0
Spring	5	0	0	0
Spring	5	0	0	0
Summer	1	0	0	656
Summer	1	0	0	44
Summer	1	0	3	622
Summer	3	0	1	220
Summer	3	0	0	160
Summer	3	0	2	102
Summer	5	0	0	125
Summer	5	0	2	15
Summer	5	0	2	2

Appendix Table 2: Abundance (n cm^{-2}) of mobile epifaunal taxa per site per season

		Species Abundance (n cm^{-2})					
Season	Site	*Palaemon peringueyi*	*Nassarius kraussianus*	*Hymenosoma orbiculare*	Unid. mollusc 1	*Assiminea ovata*	*Paridotea ungulata*
Winter	1	0	0	0	0	10	0
Winter	1	0	0	0	0	0	0
Winter	1	0	0	0	0	0	0
Winter	3	7	0	0	0	0	0
Winter	3	0	0	0	0	0	0
Winter	3	0	0	0	0	0	0
Winter	5	0	0	0	18	0	0
Winter	5	0	0	0	0	0	0
Winter	5	0	0	0	0	0	0
Spring	1	0	0	0	0	8	0
Spring	1	0	0	0	0	5	5
Spring	1	0	0	0	0	0	0
Spring	3	0	0	0	0	0	0
Spring	3	0	0	0	6	0	0
Spring	3	0	6	0	0	0	0
Spring	5	0	0	0	0	0	0
Spring	5	0	0	0	0	0	0
Spring	5	0	0	0	0	0	10
Summer	1	0	0	0	0	0	0
Summer	1	0	0	0	0	0	0
Summer	1	0	0	0	0	0	0
Summer	3	0	7	0	0	0	0
Summer	3	0	6	0	0	0	0
Summer	3	0	0	0	0	0	0
Summer	5	8	0	0	0	0	0
Summer	5	0	0	10	10	0	0
Summer	5	0	8	0	0	0	0