Demersal fish distribution in the shallow marine nearshore and estuarine seascape of Algoa Bay: Nursery areas and the effect of environmental drivers

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By

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Abstract

Estuaries and shallow marine nearshore areas are highly productive and valuable ecosystems, which provide numerous habitats for fish and support fundamental ecological links with other environments. Assessing fish distribution across estuarine and marine nearshore habitats is important to identify ecologically important habitats and develop effective management strategies for coastal fishes, many of which are important fishery species. Despite this, only a few studies have focussed on fish community patterns across an estuary and marine nearshore gradient concurrently, particularly including early life history stages, to determine the nursery value of both environments, and to examine whether these two coastal environments have distinct fish assemblages in relation to physical factors. The main aim of this study was to assess the environmental drivers of demersal fish communities in soft-bottom benthic habitats in two permanently open estuaries and adjacent marine nearshore areas (5 – 10 m) of Algoa Bay, South Africa, as well as to assess the relative roles of these two habitats as settlement and nursery areas for demersal fish species. A 1.5 m, conical shoeless beam trawl net was used to sample the demersal fish community concurrently in each habitat between July 2017 and September 2019. Sampling was conducted in July 2017, February, March, May, July, August, October and November 2019 and February, April and September 2019. DNA barcoding was used to verify identification of the early life history stages of fish caught in the estuarine and marine nearshore areas of Algoa Bay. In addition, since the two estuaries (Swartkops and Sundays) are heavily polluted, the effect of low dissolved oxygen and hypoxia and associated shifts in spatial distribution of demersal species was investigated.

The two sampled estuaries had a higher abundance of demersal fishes, with a total of 6437 fishes (28 species) caught (3752 and 2685 individuals with 24 and 20 species recorded in the Sundays and Swartkops estuaries, respectively). Species richness was higher in the marine nearshore of Algoa Bay, with 29 species (797 individuals) caught. Of the 7234 individuals caught, the identification of 100 specimens, in either a larval or early juvenile phase, were uncertain and therefore DNA barcoding was used to verify their identification. Of these 100 individuals, 86 were positively identified to species level using COI sequences. Fourteen failed to amplify by PCR and could only be identified morphologically. The marine nearshore sites were dominated by species which spawn in the marine environment and are not dependent on estuaries (marine species and marine estuary-opportunists), whilst the estuaries were dominated by estuarine spawners or marine spawners dependent on estuaries to some degree. Two discrete demersal fish assemblages were identified representing the marine nearshore and the estuary, with no significant differences observed between the two estuaries (Sundays and Swartkops). The differences observed between the marine nearshore and estuary were mostly driven by salinity, turbidity, silt and organic content of the sediment. These distinct fish assemblages might be considered as indicators for the respective environments they inhabit.

Both habitats were dominated by early life history stages (larvae to juveniles), indicating the nursery function of both habitats. Early life stages collectively comprised 97% of the catch in the marine nearshore and 68% in the estuary. Young-of-the-year (YOY) juveniles (< 1-year-old juveniles) and transformation stages (when changes in body shape and pigment pattern occur) dominated the total catch in the marine nearshore, while YOY juveniles dominated the estuarine fish assemblages. Ariidae *Galeichthys feliceps,* Haemulidae *Pomadasys olivaceus,* Sciaenidae *Argyrosomus inodorus* and Cynoglossidae *Cynoglossus zanzibarensis,* comprised the largest proportion of YOY juveniles in the marine nearshore. The transformation stage in the marine nearshore was numerically dominated by *P. olivaceus* and *G. feliceps*. In the estuarine environment, YOY juveniles were mostly dominated by Sparidae *Rhabdosargus*

holubi, Soleidae *Heteromycteris capensis* and Gobiidae *Caffrogobius gilchristi*. The greatest abundance of early life stage fishes was observed in the lower reaches of the Sundays Estuary and the upper reaches of the Swartkops Estuary, as well as nearshore sites located in close proximity to the estuary mouths, particularly during spring and summer.

Despite the fact that these coastal ecosystems are important nursery areas, they are threatened by a number of factors, including habitat loss and modification due to urban development, intensification of agriculture and subsequent eutrophication, climate change, and overfishing, all of which reduce ecosystem functioning and reduce the ecological and economic value of these habitats around the world. Hypoxia is one of the major threats to the functioning of coastal ecosystems, particularly estuaries. The Sundays and Swartkops estuaries both experience persistent eutrophic conditions, with frequent phytoplankton blooms (> 20 µg Chl-*a* l^{-1}) that result in instances of bottom water oxygen depletion (< 4 mg/l). During the present study in the Sundays Estuary, low oxygen waters were recorded in the middle reaches (Site S5) mostly during summer (four months of low DO conditions). In the Swartkops Estuary, low dissolved oxygen was recorded in the upper reaches during spring. The lowest dissolved oxygen concentration recorded was 0.5 mg/l and 2.4 mg/l in the bottom waters of the Sundays and Swartkops estuaries, respectively. Selected dominant species were only absent from areas where dissolved oxygen was < 1 mg/l and present in the adjacent sites (for example Site S4, and S3) where DO was higher mostly during January 2019. As such, the low dissolved oxygen concentrations recorded in the Swartkops Estuary did not have a noticeable impact on fish distribution, although the total abundance of species did show a slight decline when dissolved oxygen was < 3 mg/l.

This study demonstrates the importance of concurrently examining estuarine and nearshore marine habitats in order to identify ecologically important habitats, which has important implications for the development of effective management strategies for coastal fish populations, particularly in the light of anthropogenic change. In addition, in order to identify nursery hotspots it is crucial to correctly identify all the species occupying these areas. As such, this study confirms the importance of also using DNA barcoding for fish identification, particularly for the early life history stages of cryptic species (e.g. *Argyrosomus inodorus* and *Argyrosomus japonicus*).

Declaration

I, Phakama Nodo, hereby declare that the information provided in this thesis is my original work and was carried out in the Department of Ichthyology and Fisheries Science, Rhodes University, under the supervision of Prof NC James, Dr A-R Childs and Dr P Pattrick. This thesis has not been submitted to any other university.

Signature: 

Date: December 2021

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This thesis is dedicated to my daughter

LILITHA KHAZIMLA NODO

Table of contents

List of figures

List of tables

CHAPTER ONE

General Introduction

1.1 Introduction

Coastal marine environments, including estuaries, surf zones, and nearshore areas, provide valuable habitats for many fish species, including commercially and recreationally targeted fishery species, as well as species important for biodiversity and ecological functioning (e.g. Beckley 1985; Bennett 1989; Harris et al. 1999; Gillanders et al. 2003). These coastal marine habitats are used by different life stages including larvae, juveniles and adults of many species for reproduction, foraging and shelter (Blaber et al. 1995; Whitfield 1998; Harris et al. 2001). Most importantly, they provide nursery areas for fish before they move into deeper waters as they grow in size (e.g. Blaber and Blaber 1980; Potter et al. 1990; Ayvazian and Hyndes 1995; Hyndes et al. 1996).

Early research into nursery provision applied the concept of nursery areas for fish to estuaries (as a whole) because the biomass and numbers (species richness) of fish in estuaries is dominated by the juveniles of marine species, which leave the estuary as sub-adults/adults (Beck et al. 2001). Within the coastal environment, estuaries are also one of the most productive ecosystems for fishes (Whitfield 2019). Marine species in estuaries are comprised of stenohaline marine stragglers (MS), which occur in the lower reaches of the estuaries and in low numbers; euryhaline marine estuarine-opportunists (MEO), which enter estuaries in large numbers during a certain stage in their life cycle, which is usually the juvenile phase and marine estuarine-dependent species (MED), which depend on these systems for survival during the critical juvenile stage of their life cycle (Potter et al. 2015). There are also estuarine species; solely estuarine species (SE), estuarine and marine species (E&M), estuarine and freshwater species (E&F), which can complete their life cycle within the estuarine environment. These species often dominate estuarine fish assemblages in terms of abundance but not in terms of species richness or biomass (Elliott et al. 2007). Freshwater species (FS) are usually found in low numbers (and low diversity) in estuaries, with their distribution mostly restricted to the low salinity upper reaches (Potter et al. 2015). The importance of estuarine nursery areas for marine species (MED) has been attributed to environmental conditions (such as warm temperatures and high turbidity) suitable for growth, abundant food sources and the provision

of refuge/habitat from predation for juveniles (e.g. Boehlert and Mundy 1987; Whitfield 1990, 1998, 2019). The distribution and abundance of these species in estuaries depends on the characteristics of the estuarine environments, i.e. whether the estuary is permanently open or temporarily open/close (Vasconcelos et al. 2015).

Work on estuarine nursery function from the 1950s moved away from estuaries as whole and started to focus on the importance of habitats within both estuaries and the marine nearshore, which support high densities of early juvenile marine species (reviewed in Beck et al. 2001). These important habitats include estuarine and marine littoral habitats (e.g. seagrass meadows, salt marshes, mangrove forests, and marine littoral areas, intertidal pools, subtidal gulleys and surf zones) (e.g. Beck et al. 2001; Franca et al. 2009; Whitfield 2019). Vegetated habitats in estuaries are known to support higher densities of early juveniles than unvegetated habitats and this is attributed to the protection offered by the structural complexity of these habitats, high food provision and refuge for larvae and juvenile species (e.g. Beck et al. 2001; Nagelkerken et al. 2000; Whitfield and Pattrick 2015).

In the early 2000s, Beck et al. (2001) came up with the nursery-role hypothesis and defined nursery habitats as habitats within nearshore estuarine and marine environments that contribute more individuals per unit area to adult populations than other habitats; as juveniles in these habitats occur at greater densities, successfully avoid predators or grow faster than in other habitats (Beck et al. 2001). Dahlgren et al. (2006) suggested the Effective Juvenile Habitat (EJH) approach, which recognises the value of particular habitats regardless of their per unit area contributions to adult populations. In this approach, areas contributing relatively fewer individuals to adult populations such as unvegetated soft-bottom habitats, but are essential in maintaining the population, particularly in years of high variability in juvenile recruitment (Kraus and Secor 2005), are recognised as EJHs. Sheaves et al. (2006) criticised the EJH approach mentioning that it was too simplistic, and the effects of scale, complexity, connectivity, resource availability, and other biotic and abiotic processes occurring within and between habitats were not considered. Sheaves (2009) suggested that multiple habitats and their associated processes connect to form the coastal ecosystem mosaic (CEM), which is important in maintaining the overall nursery function of coastal habitats.

Recently, there has been a further shift in our understanding of nursery habitats to focus on a broader seascape nursery, a concept which includes dynamic processes as well as seascape

features that connect various habitat patches (Boström et al. 2011; Nagelkerken et al. 2015; Litvin et al. 2018). This view considers connected mosaics of different habitat patches or patches of the same habitat, with "hotspots" of high juvenile abundance identified as core areas within the seascape nursery (Nagelkerken et al. 2015; Olds et al. 2016). Litvin et al. (2018) highlighted that this approach should be the foundation for developing parameters that integrate the additional mechanisms functioning on the seascape scale that elicit nursery value.

This approach also focuses on multiple early-life stages of an individual, including transient settlement stages (Nagelkerken et al. 2015). Many coastal fishes have complex life cycles, composed of a pelagic larval phase and a benthic settlement stage (Cuadros et al. 2017). This period represents a significant challenge to settlement stage larvae as it is during this phase when the most critical morphological, physiological and behavioural transitions occur (Keefe and Able 1994). During settlement stages, settling larvae inhabit the first available suitable areas they come across when entering nursery habitats from the open marine waters (Grol et al. 2011; Nagelkerken et al. 2015). These habitats may be occupied only briefly but can still form population bottlenecks for early settlement stage fishes (Nagelkerken et al. 2015). The importance of these transient habitats is easily overlooked due to the small size at which settlement stage larvae occupies these habitats and the relatively short occupancy duration (Nagelkerken et al. 2015). The necessity for settling larvae to locate habitats during settlement stage highlights the importance of evaluating habitat features that have an effect on settlement and migration towards suitable nursery areas (e.g. Montgomery et al. 2001; Atema et al. 2002).

Understanding the functional significance of the nearshore estuarine and marine ecosystems alone and relative to each other as settlement and nursery areas requires sampling different life stages of fish concurrently from the head of the estuary to the marine nearshore environment (e.g. Valesini et al. 1997; Able et al. 2011). However, relatively few studies have taken a broader seascape approach when comparing estuarine and marine nearshore ecosystems or in the identification of core nursery habitats (critical nursery areas) for marine fish assemblages. The limited research using this approach could be linked to sampling gear limitations due to the fact that not one single sampling gear can be used to sample across multiple reaches and habitats of estuaries and adjacent nearshore habitats. Focussing on demersal fish assemblages in soft-bottom habitats of the estuarine and marine nearshore environment allows researchers to compare one specific habitat type across estuarine and nearshore marine ecosystems. The demersal fish community structure in coastal habitats is dominated (species richness) by

numerous species, which utilise coastal areas as nurseries and/or feeding and spawning grounds (Rogers et al. 1998), with adults found in deeper habitats (Gillanders et al. 2003).

In the northern Hemisphere, where researchers have sampled demersal fish assemblages from estuaries to sheltered marine embayments, many fish species were found to utilize both the estuary and the marine nearshore as settlement or juvenile habitat (Able 2005; Able et al. 2006; Woodland et al. 2012). Able et al. (2011) suggested that to determine habitat use patterns for early-life stages (late-stage larvae to juveniles) there is a need to examine patterns across the estuary – ocean ecotone. Although these studies highlighted the importance of an estuary/marine comparison to better understand the nursery role of estuarine and nearshore marine habitats for coastal fishes (Able 2005; Able et al. 2006; Woodland et al. 2012), no study has directly compared the density of different life stages within these two ecosystems or spatial patterns by life-stage across these two ecosystems (estuary and nearshore) to determine core settlement and nursery areas.

1.2.2. The nursery function of estuarine and nearshore marine ecosystems in South Africa

The nursery function for marine fish species in South Africa was assessed in the 1980s and 1990s in detail, with studies focussed on the southeast coast (Wallace et al. 1984a). This research used a variety of sampling gear and focused on juvenile fish in littoral habitats in estuaries, the surf zone, rock pools, gullies, and early-stage larval fish in the pelagic zone of the marine nearshore (reviewed in Whitfield 2019), and soft-bottom habitats in the marine nearshore (Beckley 1984; Wallace et al. 1984a). From this work, 155 species were classified as estuary-associated, and of those, over 61 species (21% of the estuary-associated taxa) are thought to use estuaries as nursery areas to some degree (Whitfield 1994; Wallace et al. 1984b). Bennett et al. (1985) even suggest that if some of these marine estuarine-dependent species (as described in Potter et al. 2015) are denied access to estuarine nursery areas, certain species might become extinct.

The high degree of dependence of marine species on estuaries as nursery areas in South Africa, contrasts with elsewhere in the southern hemisphere. For example, in temperate Australia the marine nearshore waters are used as an alternative nursery area by various estuary-associated marine species owing to the protection provided by this area (Lenanton et al. 1982; Lenanton and Potter 1987; Potter et al. 1990). This is linked to the fact that the coastline of Australia has

many large marine embayments and fringing reefs that provide alternative nursery habitats for marine juvenile species (Loneragan et al. 1989; Potter and Hyndes 1994). The sheltered embayments associated with large estuaries also serve as an alternative nursery area for many estuary-associated marine species in the northern hemisphere (Weinstein 1985). In South Africa, the coastline is more exposed and characterized by high wave action and strong ocean currents (McLachlan et al. 1981, Beckley 1985).

Subsequent research in South Africa has focussed extensively on the importance of different vegetated habitats, such as eelgrass/seagrass meadows, mangroves and salt marshes as nursery areas for fishes (e.g. Edworthy and Strydom 2016; Pollard et al. 2017; Whitfield 2017; James et al. 2019; Keur et al. 2019). These structurally complex habitats play an important role in the density and survival of early life history stages of fishes (e.g. reviewed in Whitfield 2017). This is attributed to the provision of shelter for survival of juveniles and abundant food sources (Paterson and Whitfield 2000; Whitfield 2017; James et al. 2019).

1.3. Motivation for the study

The nursery areas of many demersal fishes, particularly the marine nearshore in South Africa, are poorly known. No studies have examined early-life stages (late-stage larvae to juveniles) of demersal fishes from the freshwater zone to the ocean in soft-bottom habitat to assess core nursery areas in both the marine and estuarine environments. In South Africa, although soft-bottom habitats have been studied in the marine nearshore environment (Beckley 1984; Buxton et al. 1984; Smale 1984; Wallace et al. 1984a) and estuaries (Harrison and Whitfield 1995; Richardson et al. 2006; Vorwerk et al. 2008; Nodo et al. 2017, 2018) no direct comparisons between soft-bottom habitats in estuaries and the marine nearshore have been undertaken to examine settlement and nursery use of the early-life stages (late stage larvae to juveniles) across the estuary-ocean seascape. Furthermore, research on the nursery role of the Swartkops and Sundays Estuary for the demersal fish assemblages is also limited, with studies focusing on the littoral fishes (e.g. Harrison and Whitfield 1990; Pattrick and Strydom 2014; Edworthy and Strydom 2016).

1.4 Research aims and objectives

The main aim of this study was to assess the relative roles of soft-bottom benthic habitats in two permanently open estuaries and the marine nearshore areas (5 – 10 m) of Algoa Bay, South Africa, as nursery and settlement areas for demersal fish species. In doing so, a seascape approach was used, and the environmental drivers of demersal fish communities were assessed. DNA barcoding was used to identify the early life history stages of demersal coastal fishes for which identification was uncertain. In addition, given the ever-increasing anthropogenic pressures facing our fish resources within coastal habitats, with the two study estuaries (Swartkops and Sundays) being heavily polluted, the effect of hypoxia and associated shifts in the spatial distribution of demersal species was also investigated.

The specific objectives of this study were to:

(i) To compare the fish assmblages identified by on-site sampling and DNA barcoding
(ii) Using a seascape approach, assess the demersal fish assemblage of the shallow marine nearshore and estuarine waters of Algoa Bay and identify key environmental drivers of the demersal fish assemblage.
(iii) Determine the nursery function of shallow marine nearshore and estuarine soft-bottom habitats for demersal fishes by identifying core habitats for settlement and other early developmental life stages in Algoa Bay.
(iv) Investigate the response of demersal fishes to low dissolved oxygen in the heavily polluted Sundays and Swartkops estuaries.

Thesis structure

This thesis is divided into seven chapters, including a general introduction (Chapter 1), a study area description and general materials and methods (Chapter 2). In Chapter 3, DNA barcoding was used to verify the identification of early life history stages of fish caught in the estuarine and marine nearshore areas of Algoa Bay. Chapter 4 and Chapter 5 focus on the demersal fish assemblage of the shallow marine nearshore and estuarine waters. In Chapter 4, an ecotone approach is used to assess the fish assemblages in estuarine and nearshore marine soft-bottom habitats and the effect of environmental variables on the demersal fish assemblages across these habitats. Chapter 5 examines the relative roles of estuarine and marine nearshore environments as nursery and settlement areas for demersal fish species. In Chapter 6, the response of demersal fishes to low dissolved oxygen in two heavily polluted estuaries is investigated. Chapter 7 is a general discussion focussing on the importance of the findings and adopting a spatial approach to conservation planning.

CHAPTER TWO

Study Site

2.1 General description of the study site

The South African coastline is 3113 km in length from the Orange River on the west coast (Atlantic Ocean) to Kosi Bay on the east coast (Indian Ocean) (Figure 2.1). The coastline has ~ 74 bays and approximately 300 estuaries (Driver et al. 2011; van Niekerk et al. 2020). The east coast is influenced by the warm Agulhas Current, while the west coast is influenced by the upwelled cold nutrient-rich waters of the Benguela Current (Lutjeharms 2006) (Figure 2.1). The transition from cold to warm water from the west to east coasts forms three major biogeographical regions, which includes the cool-temperate, warm-temperate and subtropical zones (Whitfield 1998; Harrison 2002) (Figure 2.1).

The study site of this present study is the nearshore waters of Algoa Bay and the two permanently open estuaries (Swartkops and Sundays) which enter the bay (Figure 2.2) and falls within the warm-temperate region on the south-east coast of South Africa (Figure 2.1). The south-east coast is well known for its unpredictable and variable weather and is influenced by the cooling of the sea (Stone et al. 1998). The maximum and minimum air temperatures recorded at the Port of Ngqura situated between the Swartkops and Sundays estuaries (Figure 2.2) during the study period (July 2017 - September 2019) were 27.1 ºC (January 2018) and 7.1 ºC (July 2017), respectively (Appendix 2.1). Rainfall shows an autumn-spring bimodal pattern along the south-east coast, with a peak during spring months (Kopke 1988). The study was conducted during a prolonged six-year drought, with the lowest monthly rainfall recorded in June 2018 (1.8 mm) and the highest in August 2019 (73.4 mm) (Appendix 2.2).

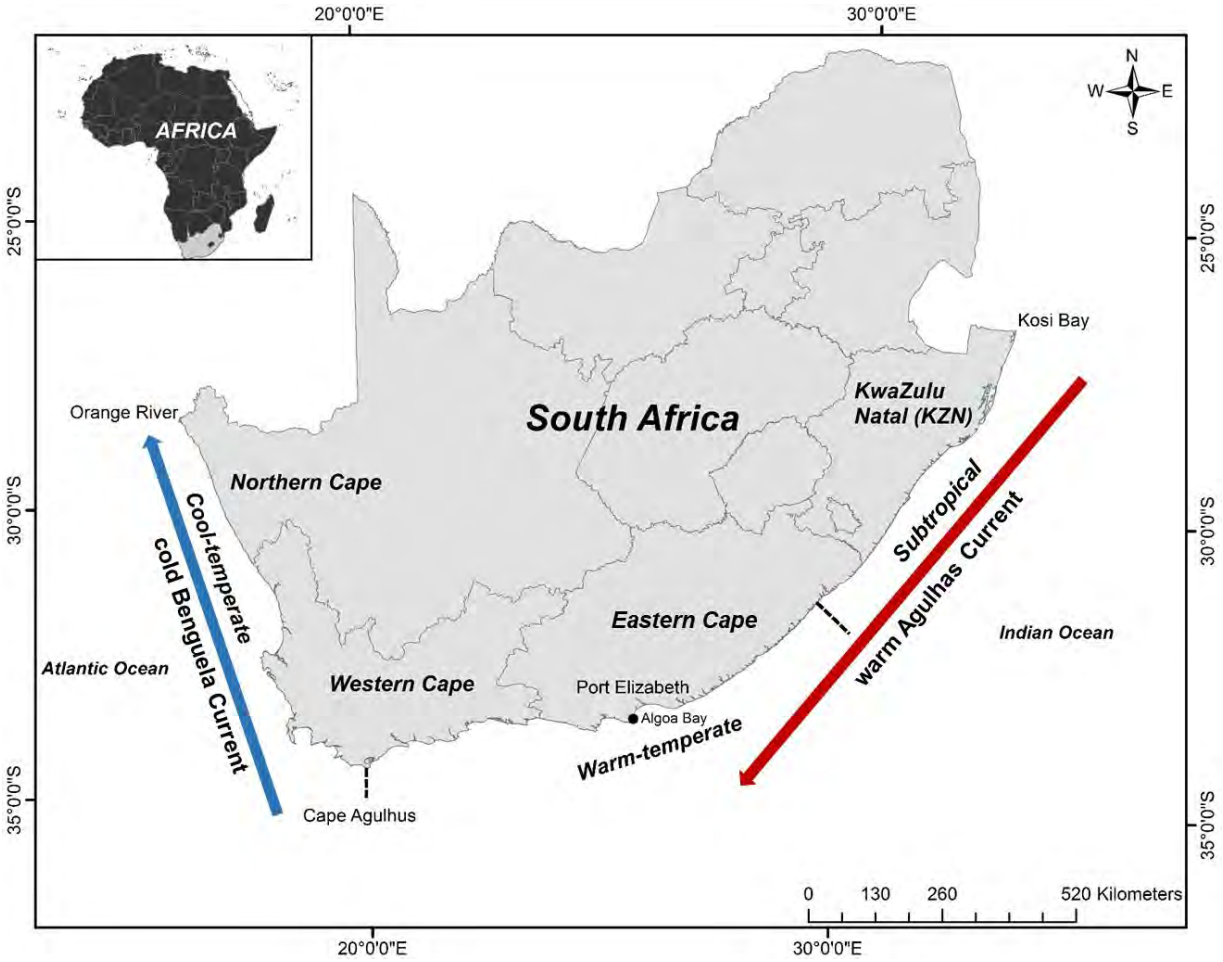

Figure 2.1: Map of South Africa showing the two major current systems, the warm Agulhas Current flowing south-westwards along the east coast and the cold Benguela Current flowing northwards along the west coast. Dashed lines divide the three major biogeographic zones (subtropical, warm-temperate, cool-temperate).

Algoa Bay is the largest (80 km between headlands) and easternmost of several log-spiral (curved) shaped embayments on the south-east coast of South Africa (Goschen and Schumann 2011). The Agulhas Current has a major influence on the bay's water circulation and structures (Schumann et al. 1995), with warm water plumes from the current occasionally infiltrating the shallow nearshore zone of the Algoa Bay (less than 15 m depth) (Roberts 2010). In the shallower waters of Algoa Bay, wind-driven, tidal, geostrophic, and inertial currents all play a part in water circulation (Goschen and Schumann 2011). Temperatures in the surface and subsurface water vary seasonally in response to seasonal variations in the bay (Goschen and Schumann 2011). Furthermore, during summer, the existence of powerful thermoclines, along with an increased frequency of easterly-component winds, promote upwelling at the eastern

(Cape Padrone) and western (Cape Recife) boundaries of the bay (Figure 2.2) (Schumann et al. 2005; Goschen et al. 2012).

Five freshwater outlets enter the bay and they vary in terms of their hydrodynamics (Figure 2.2). The major freshwater input into the bay is provided by the two permanently open estuaries, Swartkops (the heavily polluted and urbanised estuary) and Sundays (the agriculturally influenced estuary). Other heavily modified systems that enter the bay include the small-canalised Papenkuils and Baakens Rivers, which are located within heavily populated areas and are impacted by industrial and residential wastewater and the Coega Estuary which is heavily modified (has been converted into a salt works) and opens into the Port of Ngqura (Lemley et al. 2019).

The Sundays Estuary, which enters Algoa Bay at 33°43'S, 25°51'E, is a permanently open system (Whitfield and Harrison 1996), which is about 21 km long and drains a 20 729 km^2 catchment area. Along its entire length, it has been described as channel-like, with steep banks (Scharler and Baird 2005), small intertidal saltmarsh (7.3%) and no submerged macrophytes (Scharler and Baird 2005). In 1987, an interbasin water transfer network (main links being the Sundays and Great Fish estuaries) was established to meet the needs of the lower Sundays River Valley's large citrus agricultural industry (Reddering and Esterhuysen 1981).

The 14 km long (from mouth to head) Swartkops Estuary enters the Algoa Bay approximately 10 km north-east of Port Elizabeth Harbour (Figure 2.2) (Marais 1982). The Elands and Swartkops rivers, which originate in the Groot Winterhoek Mountains, supply the Swartkops Estuary with freshwater (Marais 1982). The lower reaches of the catchment and the majority of the estuary are heavily populated (Scharler and Baird 2003). The extensive mudflats, shallow creeks, and saltmarsh areas in the estuary provide refuge for invertebrates, fishes, and other ecosystem services (Baird et al. 1988).

Sampling was conducted in the shallow nearshore (< 10 m) marine environment of Algoa Bay and in the channel of the Swartkops and Sundays estuaries (Figure 2.2). In the marine nearshore environment, seven sites beyond the breakers at depths ranging from 5 to 10 m were selected (Figure 2.2). Nine sites in the Sundays Estuary (~ 2 – 3 km apart) and seven sites in the Swartkops Estuary (2 km apart) were sampled (Figure 2.2). The three environments (Algoa Bay marine nearshore, Sundays and Swartkops estuaries) were sampled on different days but

within the same week. Sampling was conducted between July 2017 and September 2019 for a total of three sampling occasions per season (12 sampling occasions). Sampling was conducted in July 2017, February, March, May, July, August, October and November 2018 and February, April and September 2019. Details of the fish sampling can be found in Chapter Three.

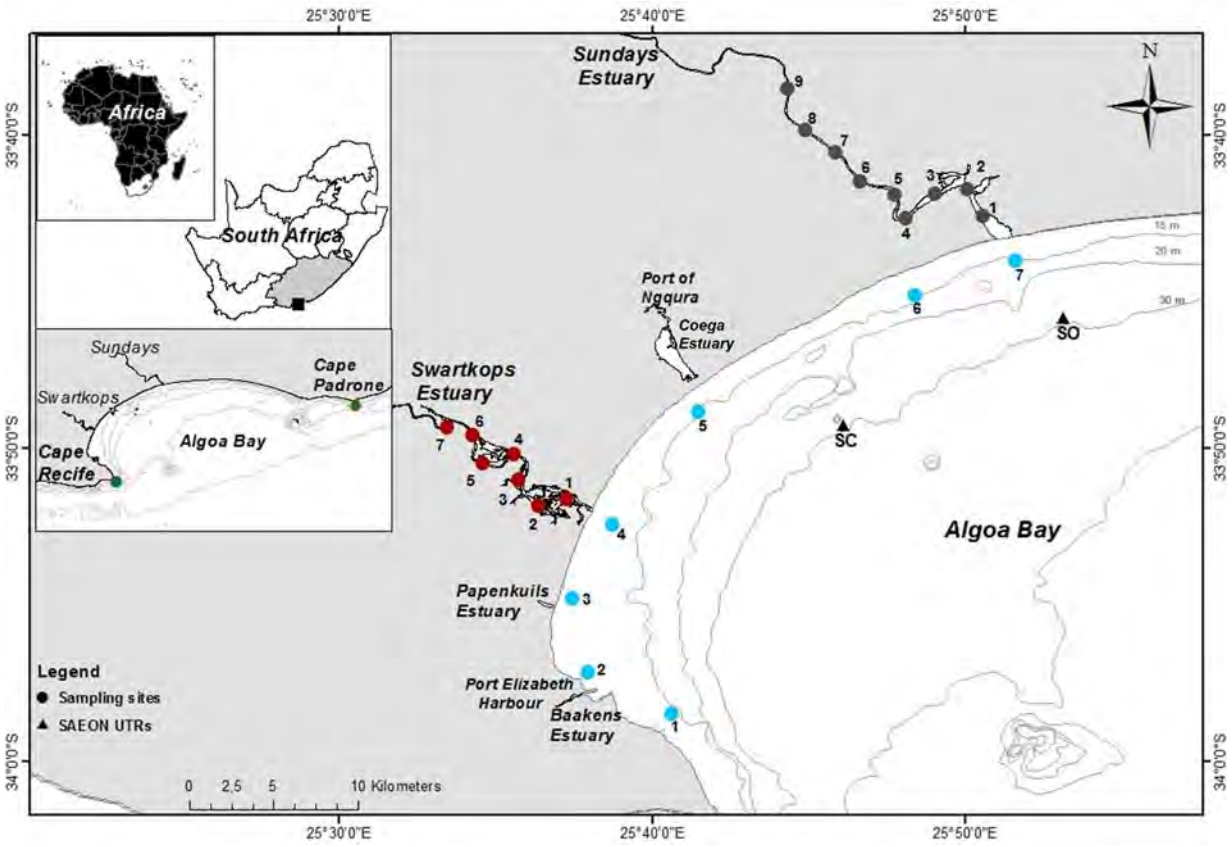

Figure 2.2: Map of Algoa Bay showing the study sites (circles) and the location of the Swartkops and Sundays estuaries as well as the South African Environmental Observation Network (SAEON) underwater temperature recorders (UTRs) (triangles). SC – ST Croix and SO – Sundays River. The blue dots represent the marine nearshore sampling sites, red dots represent Sundays Estuary sampling sites and the grey dots represent Swartkops Estuary sampling sites.

2.2. Physico-chemical characteristics of the study area

Physico-chemical parameters recorded at the time of sampling included water temperature (ºC), salinity, turbidity (NTU) and dissolved oxygen (mg/l) using a YSI (6290) multi-parameter probe where both surface and bottom waters were measured. At each site, sediments were collected seasonally (once in each season for a total of four months) using a cone dredge for organic content analysis. In February 2020, sediment was also collected at each site using a

cone dredge for particle size distribution. Sediment samples from each site were stored in plastic containers (350 ml jar) in a cooler box with ice for further analysis in the laboratory.

Each sediment sample was subsampled to determine sediment organic content and sediment particle size. To determine sediment organic content approximately 20 g of the sediment sample was placed in a container (crucible) of known weight, weighed and then heated in the oven (Labcon-model number: FSOMB) to 65 ºC for 24 hours to dry. The dried sediment was placed in a desiccator to cool down before weighing. After cooling, dry sediment samples were then weighed to obtain the dry weight and then further heated to 760 ºC in a furnace (Lenton) for 24 hours and then weighed again to obtain ash-free dry weight. The percentage of organic content was then calculated using the following formula (Briggs 1977; Heiri et al. 2001):

$$Organic\ content\ (\%) = \frac{Dry\ weight - Ashed\ weight}{Dry\ weight} \times 100$$

To determine sediment particle size, wet sediment sampled was oven dried and 50 g of dry sediment was passed through the sieves of different mesh sizes including 2000 µm, 1000 µm, 500 µm, 355 µm, 250 µm, 180 µm, 125 µm, 90 µm and 63 µm and a pan. The weight retained by each sieve was determined and the particle size distribution calculated from the weight retained in each sieve. Using the Wentworth scale (Wentworth 1922; Folk 1965) samples were then classified into gravel (> 2000 µm), fine gravel (1000 – 2000), course sand (500 – 1000 µm), medium sand (355 – 500 µm), fine sand (180 – 250 µm), very fine sand (90 – 125 µm), very fine sand (63 – 90 µm) and silt/mud (< 63 µm).

Principal Component Mean Analysis (PCA) in PRIMER v7 (Plymouth Routines in Multivariate Ecological Research) package (Clarke and Warwick 2001) was used to determine if sites in the marine nearshore environment and the two estuaries could be grouped according to environmental characteristics (salinity, temperature and turbidity of the bottom water, along with silt, sand and organic content of the sediment). Draftsman plots were generated to see if there were any correlations between variables and to determine if transformation was needed for any environmental variable (Clarke and Gorley 2015). Organic content (in percentage) and silt (in percentage) were not normally distributed and were log transformed for this analysis. All variables were then normalised (raw data value–mean/ max–min) to transform variables to equal scales (Clarke and Warwick 2001). One-way analysis of similarity (ANOSIM) was

conducted to determine if there were significant differences among estuarine reaches, and between the sheltered and exposed sites in the Algoa Bay marine nearshore based on the wave data. This analysis was conducted in PRIMER v7 (Plymouth Routines in Multivariate Ecological Research).

2.2.1. *Habitat characterization and site classification*

Algoa Bay

To classify substratum type and characterizing the main/dominant sediment type, images of the seafloor were only collected at each nearshore marine site using a drop camera system consisting of two GoPro Hero 7 black cameras housed in a rectangular stainless steel (Figure 2.3). During each drop, the camera was configured to perform interval photography, which automatically captured a picture every five seconds. The camera was left on the substratum for approximately one minute to allow disturbed sediment to settle. Several photos of the same section of seabed were captured and one clear image was chosen from each site for processing.

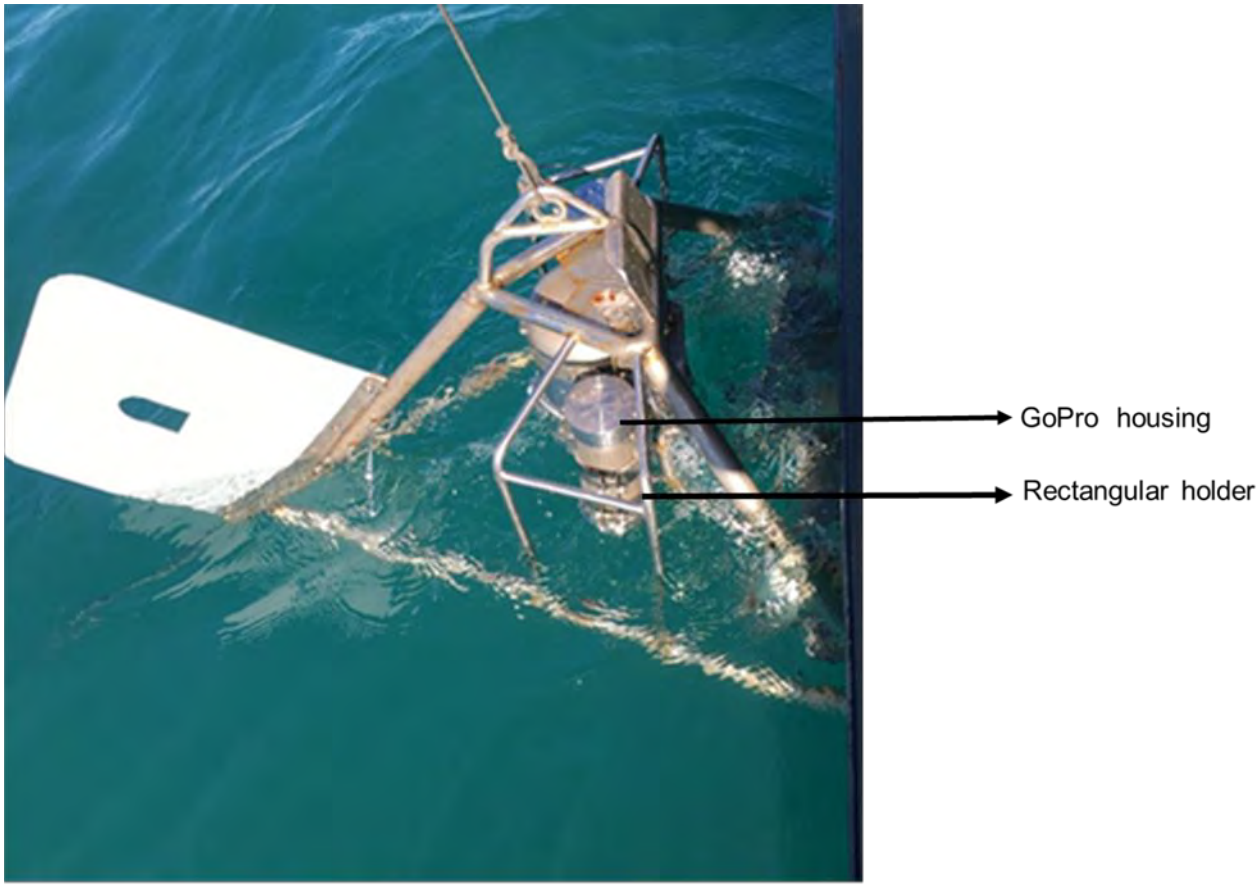

Figure 2.3: Drop camera system consisting of two GoPro Hero 7 cameras housed in a rectangular stainless steel holder.

Of the seven site images processed, three sites (A4, A5 and A7) were identified as dominated by sand with three other sites (A1, A2 and A6) identified as dominated by reef-sand and one site (A3) dominated by sand-mud (Table 2.1, Figure 2.4).

Table 2.1: The Algoa Bay marine nearshore substratum type characterizing the main/dominant sediment type for each site visualized with a drop camera during the sampling period (August 2019).

Site	Substratum type
A1	Reef-sand
A2	Reef-sand
A3	Sand-mud
A4	Sand
A5	Sand
A6	Reef-sand
A7	Sand

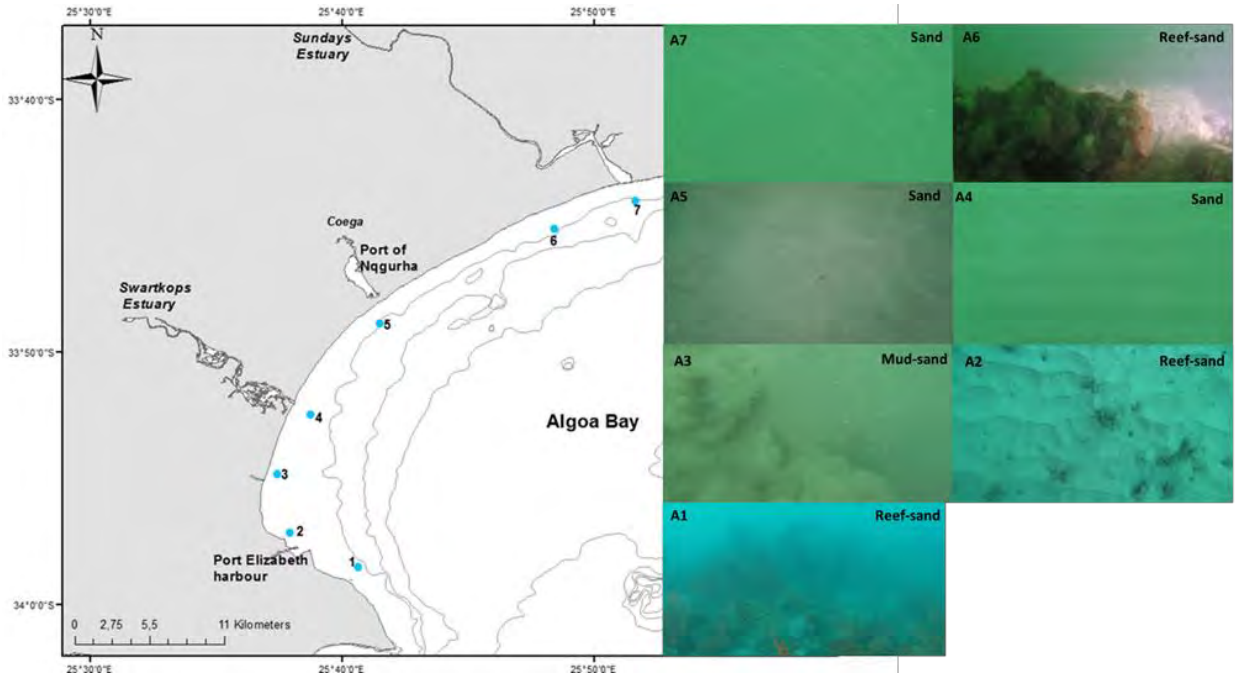

Figure 2.4: Drop camera images representing substrate type characterizing the main/dominant sediment type of each site (A1 to A7) in Algoa Bay during the sampling period (August 2019).

The wave heights were consistently higher in the eastern sector of the bay which includes the area offshore of the Sundays Estuary (Site A7) (Figure 2.5). The western sector of the bay, which includes the area nearshore of the Swartkops Estuary mouth (site A3 and A4), however tends to be more sheltered (Figure 2.5).

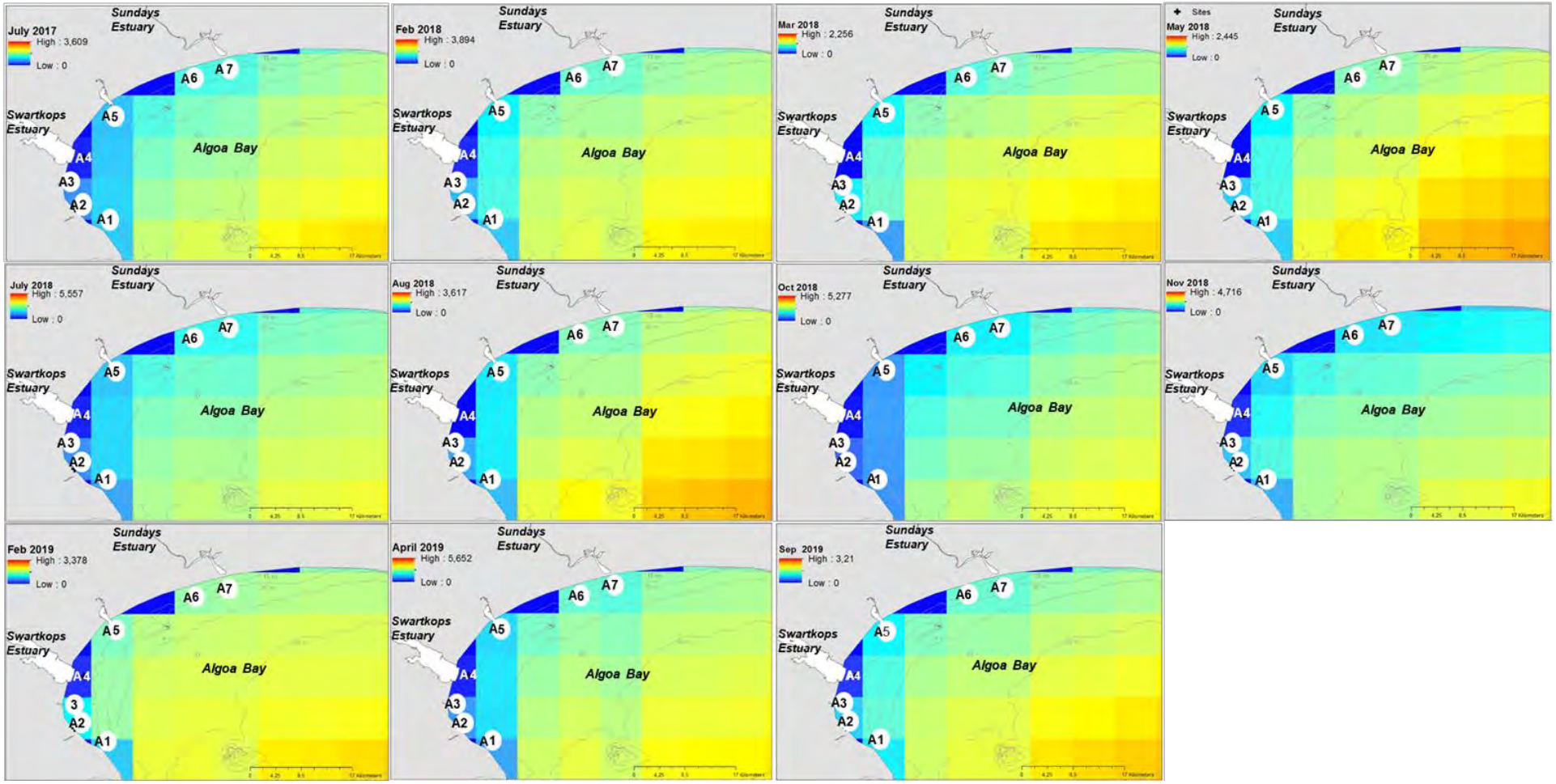

Figure 2.5: Spatial changes in significant wave heights in the Algoa Bay marine nearshore on the days sampled with one month missing (owing to a missing data in January 2019). Data were obtained from the South African Weather Service (SAWS).

The PCA ordination in the Algoa Bay marine nearshore, the first two axes accounted for approximately 57% of the variation among sites (Figure 2.6). Although, ANOSIMI showed that the sheltered sites were not significantly different from the exposed sites (R = 0.13; P = 0.02), PCA ordination in the Algoa Bay marine nearshore showed the separation of some sites from the exposed sites (Figure 2.6).

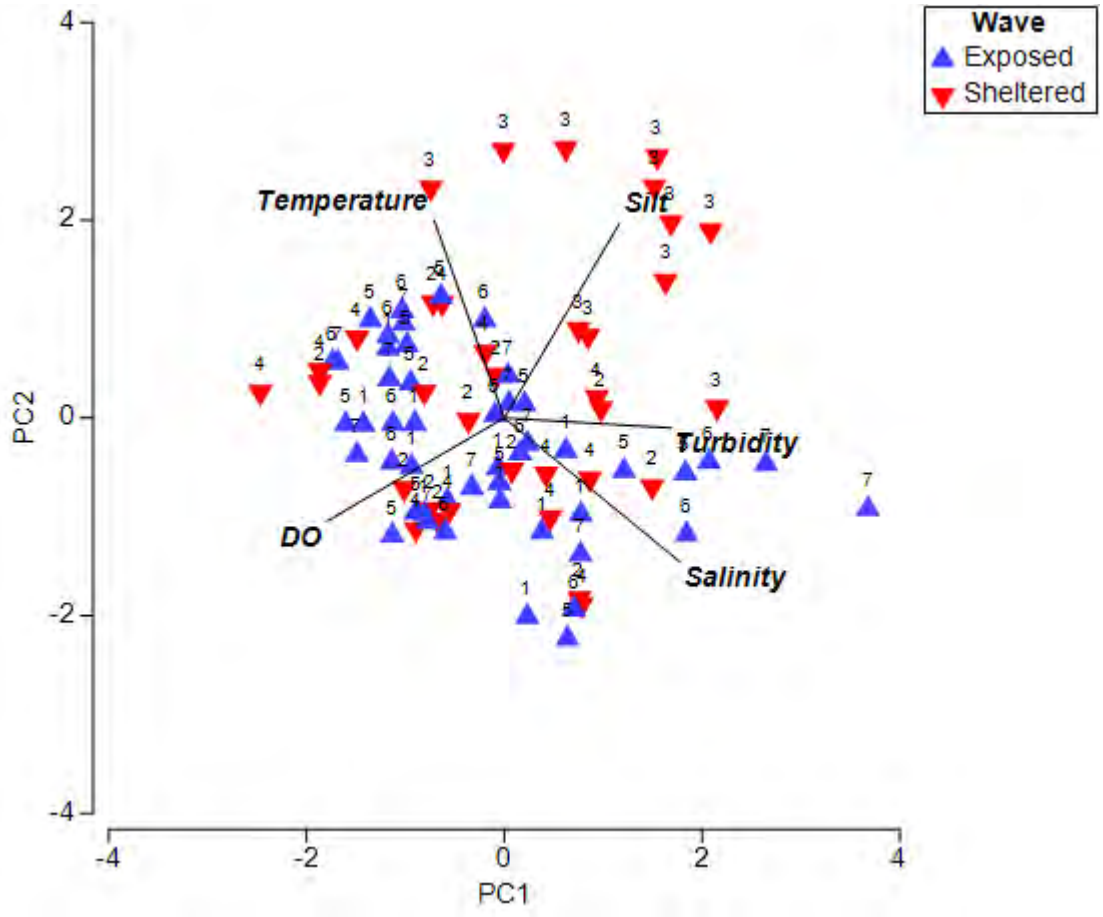

Figure 2.6: Principal component analysis (PCA) showing the grouping of the exposed and sheltered sites (wave height) based on bottom water temperature (ºC), salinity, turbidity (NTU) and silt (%) recorded during the study period (July 2017 – September 2019).

Sundays Estuary

The PCA ordination in the Sundays Estuary, divided the sites based on salinity (-0.15), dissolved oxygen (0.19), silt (0.52) (PC 1), organic content (0.13), temperature (-0.49) and turbidity (-0.48) (PC 2). The first two axes accounted for approximately 59% of the variation among sites (Figure 2.7). The PCA ordination in the Sundays Estuary showed that Sites S1 and S2 in the lower reaches were characterised by high salinity, low temperature, low turbidity, high DO, low organic content and high silt percentage. Site S3 to Site S6 in the middle reaches were characterized by high organic content and low silt percentage. Sites S7 to Site S9 were in the upper reaches and were characterised by low organic content, high silt, high temperature, high turbidity, low DO and low salinity (Figure 2.7). There were highly significant differences among estuarine reaches (ANOSIM; R = 0.5, P = 0.001), with sites in the lower reaches significantly different from both middle (R = 0.3, P = 0.001) and upper (R = 0.7, P = 0.001) reaches and sites in the middle reaches significantly different from the upper reaches (R = 0.6, P = 0.001).

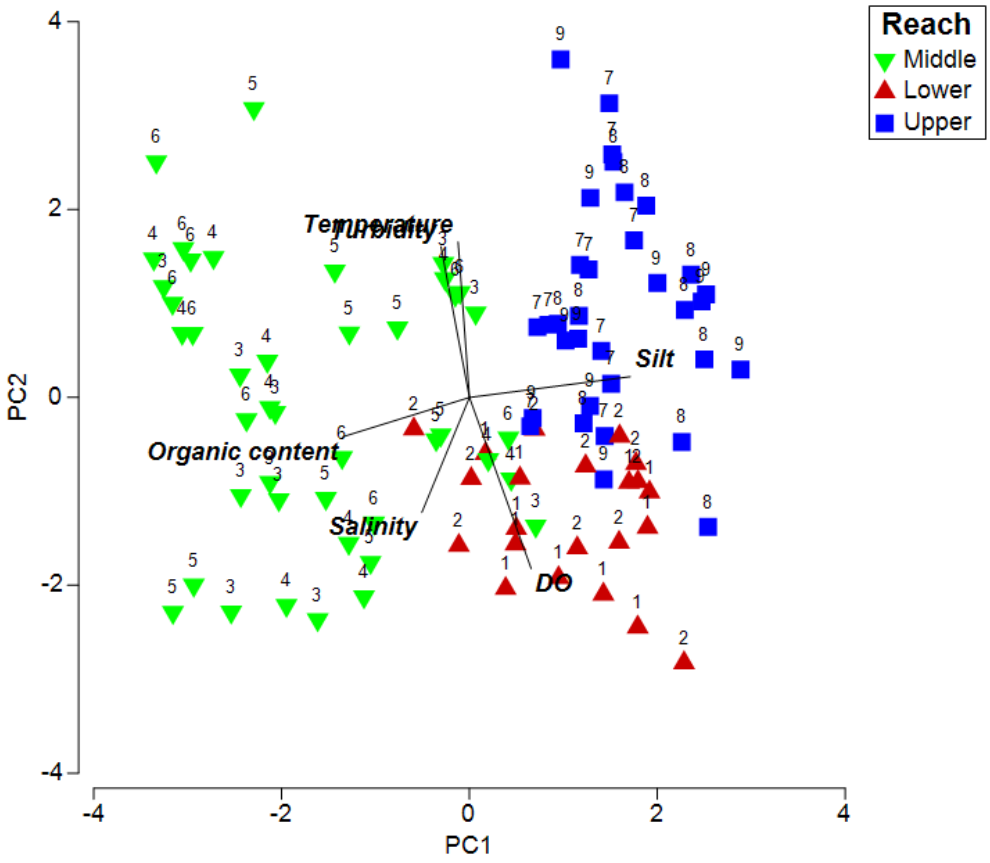

Figure 2.7: Principal component analysis (PCA) showing the lower, middle and upper reaches of the Sundays Estuary based on mean bottom water temperature (ºC), salinity, turbidity (NTU), organic content, dissolved oxygen and silt (%) recorded during the study period (July 2017 – September 2019).

Swartkops Estuary

The PCA ordination in the Swartkops Estuary, divided the sites based on salinity (-0.21), silt (-0.59-), dissolved oxygen (-0.28) (PC 1), organic content (0.07), turbidity (-0.43) and temperature (-0.41) (PC 2). The first two axes accounted for approximately 63.7% of the variation among sites (Figure 2.7) (Figure 2.8). Sites SW6 and SW7 in the upper reaches were influenced by low salinity and low dissolved oxygen (DO), high turbidity and high temperature. The lower reaches comprised of sites SW1, SW2 and SW3 and were associated with high salinity and high dissolved oxygen, low turbidity and low temperature. Sites SW4 and SW5 were associated with intermediate conditions (Figure 2.8). Several smapling occasions for SW2 had high organic content (and low silt). Although there were significant differences among these estuarine reaches (ANOSIM: R = 0.4, P = 0.001), sites in the middle reaches were not significantly different from the lower reaches (ANOSIM: R = 0.2; P = 0.002) and the upper reaches (ANOSIM: R = 0.1; P = 0.002).

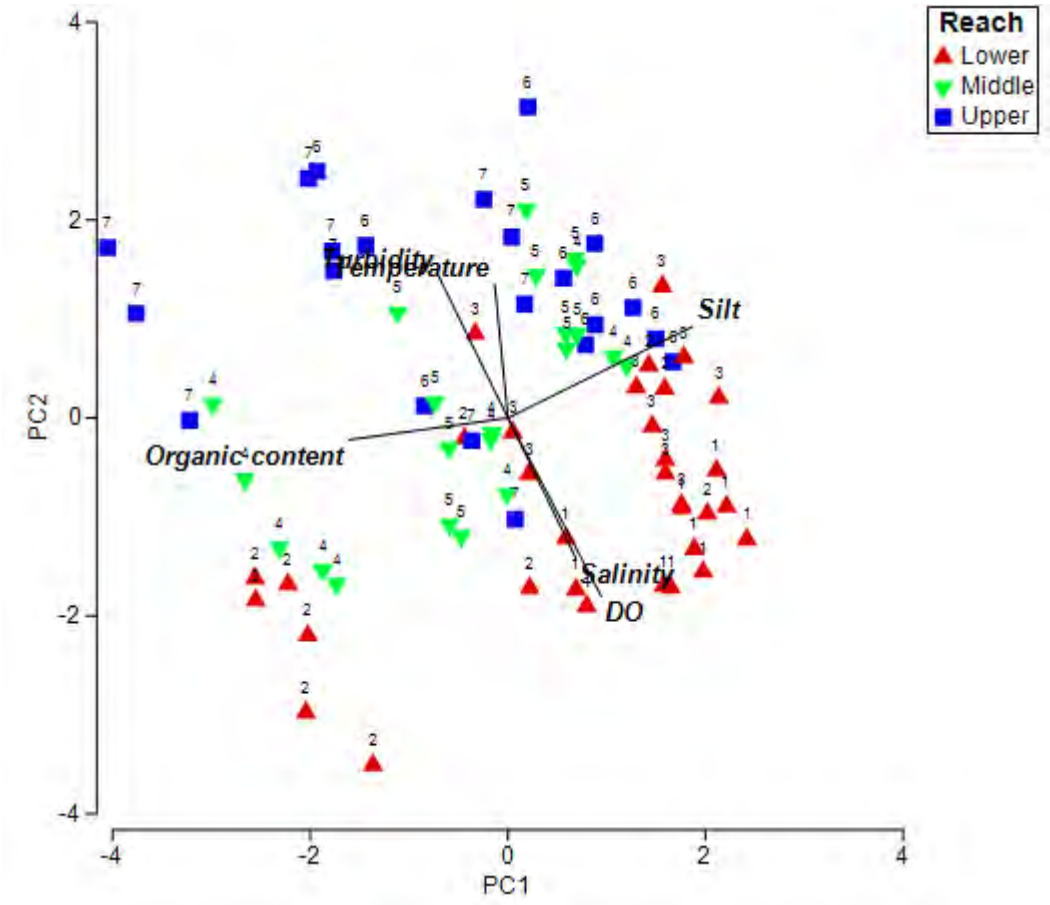

Figure 2.8: Principal component analysis (PCA) showing the lower, middle and upper reaches of the Swartkops Estuary based on mean bottom water temperature (ºC), salinity, turbidity (NTU), organic content and silt (%) recorded during the study period (July 2017 – September 2019).

2.2.2. *Algoa Bay marine nearshore environment*

In the marine nearshore environment, sediment was comprised mainly of sand (80 – 98%) at all sites, with a small component of silt/mud (0.05 to 0.8%). The silt/mud component was highest at Site A3 (13%) (Figure 2.9a. Appendix 2.6). The organic content in the sediment of the Algoa Bay marine nearshore sites was fairly similar (0.4 ± 0.04 – 0.7 ± 0.4%) in all sites except for Site A1 where the overall mean percentage was considerably higher (3.6 ± 5.5%) than other sites in winter (Figure 2.9b; Appendix 2.3).

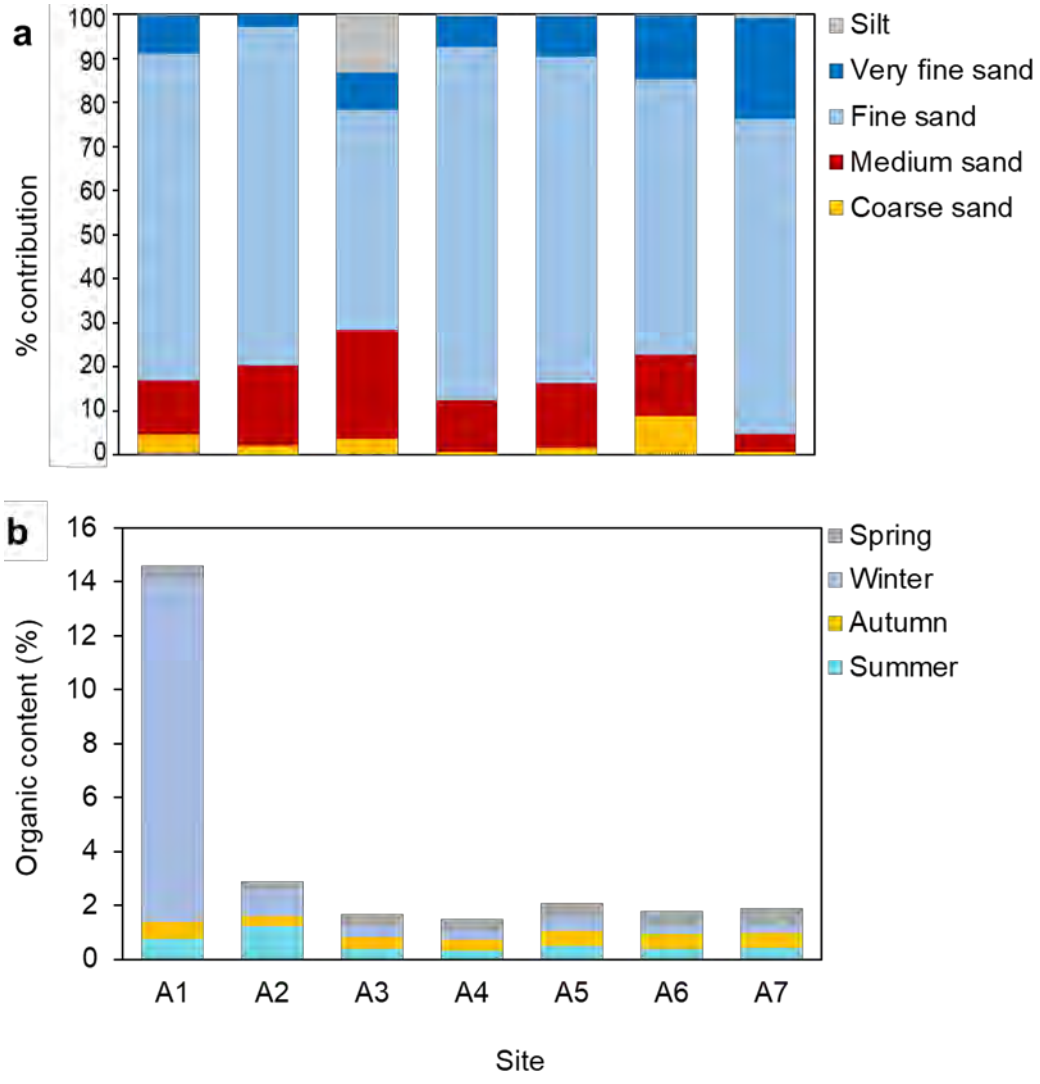

Figure 2.9: The (a) sediment particle size composition and (b) organic content in Algoa Bay Marine nearshore during the sampling period (July 2017 – September 2019).

In the marine nearshore, there was not much variation in salinity among sites, with salinity ranging from 34.1 – 36.4 (Site A4) to 34.5 – 36.6 (Site A2, A5 & A6) at the bottom (Table 2.2, Appendix 2.4). Bottom water temperatures were higher (15.8 – 23.3 ºC) at Sites A3 and A4 than other sites. Water turbidity was higher at the sites closer to the estuary mouths and Papenkuils Outlet (A3, A4, A6 & A7) than other sites at the bottom. Turbidity ranged from 0.3 – 22.2 NTU (Site A4), 0.3 – 28.8 NTU (Sites A1 & A2) and 0.3 – 52 NTU (Site A7) (Table 2.2, Appendix 2.5). The lowest dissolved oxygen was recorded at Site A7 (4.4 mg/l) and the highest was recorded at A4 (10 mg/l) (Table 2.2, Appendix 2.5) at the bottom.

Table 2.2: Mean and range of physico-chemical parameters measured in the surface (S) and bottom (B) waters of the Algoa Bay marine nearshore during the study period (July 2017 – September 2019).

Site	Depth (m)	Salinity		Turbidity (NTU)		Temperature (°C)		Dissolved oxygen (mg/l)	
		S	B	S	B	S	B	S	B
1	6	35.2(34.4 - 36.6)	35.2(34.3 - 36.5)	1.1(0.1 - 3.5)	1.9(0.3 - 8.4)	18.8(15.9 - 21.4)	17.2(15.9 - 22.3)	7.9(7.8 - 9.3)	7.6(6.1 - 8.7)
2	6	35.2(34.6 - 36.6)	35.2(34.4 - 36.6)	1.1(0.1 - 3.7)	1.9(0.1 - 10.4)	19.5(15.4 - 22.1)	18.1(15.3 - 21. 4)	7.8(6.6 - 9.4)	7.3(5.6 - 9.3)
3	6	35.1(34.5 - 36.5)	35.3(34.5 - 36.5)	3.1(0.2 - 13.5)	7.6(0.6 - 9.8)	19.6(16.0 - 22.2)	18.2(15.8 - 23. 3)	8.4(6.8 - 11.8)	6.6(5.1 - 8.7)
4	5	35.2(34.3 - 36.4)	35.1(34.1 - 36.5)	1.1(0.1 -4.3)	7.4(0.3 - 22)	19.3(15.9 - 21.8)	18.9(15.8 - 23.3)	8.6(7.3 - 10.5)	7.9(6.5 - 10.3)
5	6	35.3(34.5 - 36.6)	35.3(34.5 - 36.6)	1.0(0.1 - 3.6)	2.8(0.2 - 10.2)	19.2(15.5 - 21.9)	18.4(15.2 - 20.9)	7.9(6.6 - 9.5)	7.6(5.8 - 8.9)
6	6	35.3(34.4 - 36.7)	35.2(34.3 - 36.6)	2.5(0.1 - 16.8	5.5(0.3 - 28.8)	18.9(15.2 - 21.7)	18.5(15.1 - 21.4)	7.7(7.0 -9.1)	7.5(5.8 - 8.4)
7	7	34.0(25.0 - 36.1)	35.3(34.4 - 36.1)	3.1(0.4 - 26.0)	7.4(0.3 - 52)	18.6(15.2 - 20.9)	18.2(15.2 - 20.22)	7.9(7.2 - 8.8)	7.5(4.4 - 9.1)

2.2.3. Sundays Estuary

In the Sundays Estuary, a horizontal salinity gradient was observed throughout the study period, with bottom salinity ranging from 27.7 – 35.1 at the mouth (Site S1) to 2.1 – 3.1 at the head of the estuary (Site S9) (Table 2.3, Appendix 2.4). Euhaline (30.0 – 39.9) conditions were restricted closer to the mouth region (0 – 3.5 km from the mouth) (S1 & S2). Polyhaline (18 – 29.9) conditions extended from 3.5 km (Site S3) to 12 km (Site S6) from the mouth. Mesohaline (5.0 – 17.9) conditions stretched from 12 km to 16 km form the mouth which included Site S7 and S8. Oligohaline (0.5 – 4.9) conditions were restricted at Site S9, which was about 21 km from the mouth (Table 2.3).

Bottom water temperature increased from Site S1 (15.9 – 22.3) to the upper most sites (S7 – S9) (15.3 – 25.3) (Table 2.3, Appendix 2.4). Turbidity at the bottom was mostly higher in the upper sites (S7 – S8) (4.5 – 32.1 NTU) and middle sites (Site S4 & S6) (4.5 – 31.5 NTU), with lowest values recorded closer to the mouth (Sites S1 & S2) (1.8 – 7.5 NTU) (Table 2.3, Appendix 2.5). According to Cyrus and Blaber's (1987) turbidity classification, this estuary can be classified as intermediate (10 – 80 NTU) based on the turbidity values recorded during the study period (Table 2.3, Appendix 2.5). In the Sundays Estuary, dissolved oxygen at the bottom was lower at Site S5 (0.5 – 10.2 mg/l) compared to other sites and consistently higher at Site S1 (7.5 – 9.1) mg/l) than the rest of the estuary (Table 2.3, Appendix 2.5).

Table 2.3: Mean and range of physico-chemical parameters measured in the surface (S) and bottom (B) waters of the Sundays Estuary during the study period (July 2017 – September 2019). Blue shading shows Euhaline (30.0 – 39.9) conditions, grey shading shows Polyhaline (18 – 29.9) conditions, yellow shading shows Mesohaline (5.0 – 17.9) conditions and light shading shows Oligohaline (0.5 – 4.9) conditions.

Site	Depth (m)	Salinity		Turbidity (NTU)		Temperature (°C)		Dissolved oxygen (mg/l)	
		S	B	S	B	S	B	S	B
1	1	28.5(17.0 - 34.6)	31.9(27.7 - 35.1)	3.9(2.0 - 10.3)	4.4(1.8 - 7.5)	19.9(17.3 - 22.4)	18.9(15.9 - 22.3)	7(6.0 -11.1)	8.0(7.5 - 9.1)
2	3	21.2(16.3 - 26.4)	30.2(24.5 - 33.9)	5.4(3.0 - 8.1)	8.8(4.2 - 20.01)	21.1(17.2 - 25.7)	18.8(15.3 - 21.4)	8.3(6.9 - 11.0)	7.8(7.1 - 9.4)
3	2	16.2(8.4 - 24.0)	27.5(18.0 - 32.3)	5.7(2.4 - 11.5)	12.6(4.5 - 31.5)	21.5(17.0 - 26.4)	19.4(15.8 - 23.3)	8.0(4.1 - 11.5)	6.2(3.1 - 9.2)
4	1	13.6(5.9 - 19.9)	24.0(13.5 -31.4)	7.2(2.7 - 15.2)	13.4(4.2 - 28.8)	21.3(16.6 - 26.4)	19.8(15.8 - 23.8)	8.5(6.2 - 11.5)	5.8(3.0 - 8.9)
5	1	9.7(4.0 - 14.7)	17.3(10.9 - 23.8)	8.9(2.3 - 18.9)	13.1(2.5 - 22.1)	21.1(16.2 - 26.8)	20.6(16.5 - 24.6)	8.8(5.6 - 12.3)	6.1(0.5 – 10.2)
6	1	6.8(3.4 - 10.9)	13.7(7 - 20.9)	10.0(2.0 - 29.5)	14.1(2.5 - 21.3)	20.8(15.8 - 26.7)	20.6(15.5 - 25.4)	6.7(5.5 - 8.4)	4.7(2.3 - 8.2)
7	1	4.1(2.4 -6.8)	6.7(2.4 - 18.9)	11.6(2.3 - 23.4)	16.6(5.6 - 31)	20.3(13.3 - 26.2)	20.7(15.3 - 25.9)	6.6(5.1 - 8.6)	5.9(4.7 - 7.4)
8	1	2.9(2.4 - 4.1)	3.5(2.4 - 7.3)	10.6(4.9 - 26.3)	12.6(4.8 - 29.8)	20.4(15.1 – 25.8)	20.4(15.1 - 25.6)	6.3(4.8 - 7.9)	6.3(3.8 - 8.8)
9	1	2.5(2.0 - 3.1)	2.5(2.1 - 3.1)	10.6(5.9 -26.9)	11.1(4.3 - 32.1)	20.1(14.7 - 25.8)	20.0(14.7 - 25.3)	6.5(3.6 - 15.3)	6.1(4.1 - 8.6)

In the Sundays Estuary, sediment comprised mostly of sand (88.1 – 97.6%) and a smaller percentage (2.4 – 11.9%) of silt/mud in all sites (Figure 2.10a, Appendix 2.6). The percentage of silt/mud was highest at S1 to S4 (5.6 – 11.9%), with the lowest values (silt/mud) recorded at S7 to S9 (2.4 – 3.0%) (Figure 2.10a, Appendix 2.6). Mean percentage organic content of the sediment ranged between 0.5 ± 0.2% at S1 and 1.7 ± 0.3% at Site S5 (Figure 2.10b, Appendix 2.3), with the lowest organic content recorded in spring across all sites (Figure 2.10b).

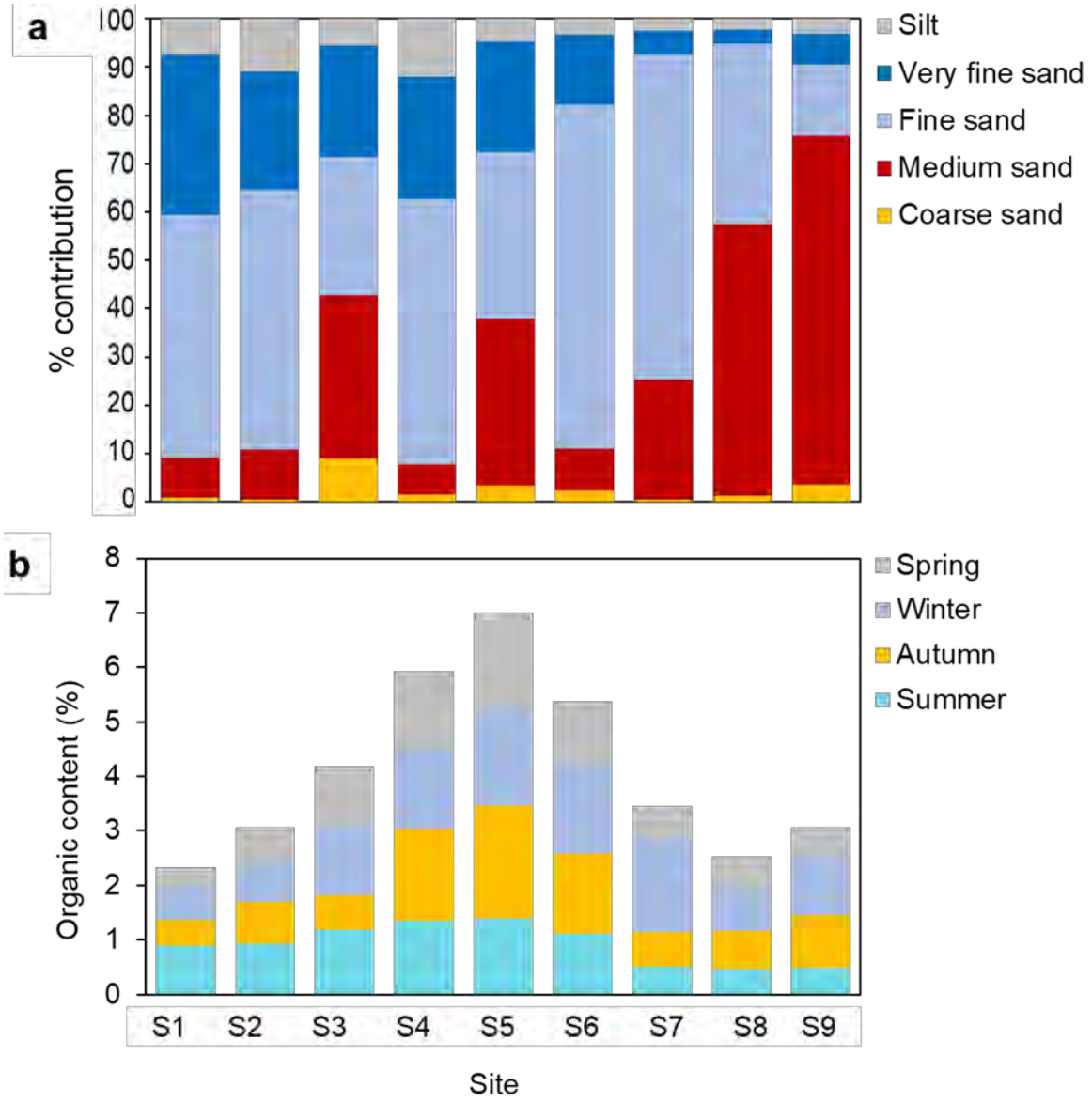

Figure 2.10: The (a) sediment particle size distribution and (b) organic content (%) in Sundays Estuary during the sampling period (July 2017 – September 2019).

2.2.4. Swartkops Estuary

In the Swartkops Estuary, bottom salinity also decreased with an increase in distance from the mouth to the head region, with salinity ranging from 18.4 – 24.8 at the uppermost sites (SW7) to 33.1 – 35.8 at the mouth region (SW1) (Table 2.4; Appendix 2.4). Euryhaline (30.0 – 39.9) conditions were recorded closer to the mouth region (0 – 4 km form the mouth). Polyhaline (18 – 29.9) conditions extended from 4 km (Site SW3) to 14 km (Site SW7) from the mouth.

Temperatures at the bottom ranged from 15.9 – 22.5 ºC at the mouth region (Site SW1) to 15.9 – 26.7 ºC at Site SW5 (Table 2.4, Appendix 2.4). Bottom turbidity increased from the mouth to the upper sites with values ranging from 0.7 – 4.6 NTU (Site SW1) to 2.3 – 30.3 NTU (Site SW6) (Table 2.4, Appendix 2.5). According to the Cyrus and Blaber (1987) turbidity classification, this estuary can be classified as clear (<10 NTU) based on the mean turbidity values recorded during the sampling period (Table 2.4, Appendix 2.5). In the Swartkops Estuary, consistently low dissolved oxygen conditions (2.5. – 4.3 mg/l) were observed at Site SW7 during the study period, with mean dissolved oxygen decreasing with an increase in distance from the mouth at the bottom. Overall dissolved oxygen ranged from 2.5 – 8.8 mg/l (Site SW7) to 7.1 – 8.3 mg/l (Site SW1) during the study period (Table 2.4, Appendix 2.5).

Table 2.4: Mean and range of physico-chemical parameters measured in the surface (S) and bottom (B) waters of the Swartkops Estuary during the study period (July 2017 – September 2019). Blue shading shows Euhaline (30.0 – 39.9) and grey shading shows Polyhaline (18 – 29.9) conditions.

Site	Depth (m)	Salinity		Turbidity (NTU)		Temperature (°C)		Dissolved oxygen (mg/l)	
		S	B	S	B	S	B	S	B
1	2	34.6(33.2 - 35.8)	34.7(33.1 - 35.8)	1.5(0.3 - 2.6)	2.6(0.7 - 4.6)	19.1(14.4 - 22.5)	19.5(15.9 - 22.5)	7.6(7.0 - 8.5)	7.6(7.1 - 8.3)
2	1	32.1(26.82 - 35.4)	30.5(28.3 - 35.4)	1.9(0.1 - 3.4)	3.3(1.6 - 7.2)	20.3(16.1 - 25.0)	20.3(16.3 - 24.9)	6.6(5.0 - 8.0)	6.7(5.1 - 8.8)
3	1	28.5(24.4 - 32.6)	28.5(24.4 - 32.9)	2.9(1.2 -5.8)	3.8(1.5 - 8.1)	21.1(16.1 - 27.4)	20.9(16.1 - 25.7)	5.9(4.4 - 7.0)	6.3(4.9 - 7.9)
4	2	25.6(22.5 - 30.0)	26.9(23 -31.4)	2.3(1.3 - 4.6)	4.5(1.3 - 12.6)	21.1(16.2 - 25.8)	20.7(16.0 - 25.5)	6.7(4.4 - 8.5)	5.9(3.9 - 6.7)
5	2	22.6(19.3 - 26.8)	24.7(21.9 - 26.9)	2.6(0.9 - 6.2)	5.5(1.4 - 14)	21.3(16.1 - 25.9)	21.4(15.9 - 26.7)	5.9(3.9 - 7.6)	5.4(3.5 - 6.8)
6	3	20.4(16.8 - 23.2)	22.8(20.4 - 24.7)	4.2(0.6 - 13.0)	10.3(2.3 - 30.3)	21.3(15.9 - 25.7)	21.1(16.2 - 25.7)	5.8(3.3 - 11.2)	5.2(3.4 - 7.5)
7	2	17.6(14.5 - 21.2)	21.6(18.4 - 24.8)	2.6(0.5 - 8.2)	9.5(1 - 20.4)	21.4(16.0 - 26.0)	21.3(16.6 - 25.6)	7.1(3.6 -15.3)	4.2(2.5 - 8.8)

In the Swartkops Estuary, sediment also comprised of sand and silt/mud, with sand dominating throughout the system (80 – 100%). The percentage of silt/mud in the sediment increased from the SW1(0.01%) to SW7(17%) (Figure 2.11a, Appendix 2.6). The highest organic content percentage was recorded at Site SW4 (0.6 – 0.8 %) and the uppermost site (SW7) with organic content increasing in summer at SW7. The lowest organic content was recorded at Site SW6 (0.1 – 0.3%) (Figure 2.11b, Appendix 2.3).

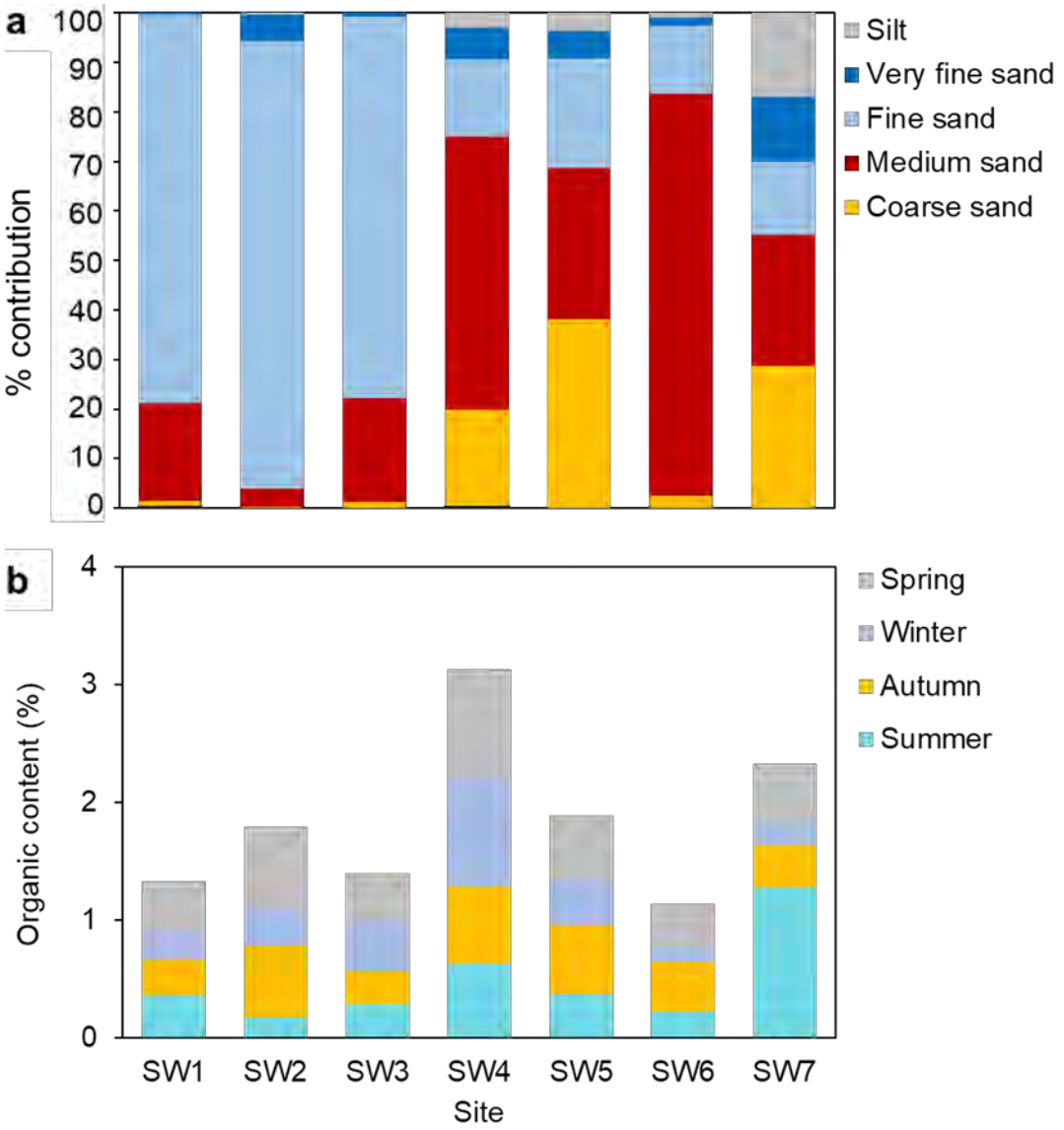

Figure 2.11: The (a) sediment particle size distribution and (b) organic content in Swartkops Estuary during the sampling period (July 2017 – September 2019)..

CHAPTER THREE

Using DNA barcoding to identify the early life history stages of demersal coastal fishes

3.1 Introduction

Accurate taxonomic identification to the species level of early life history stages of fishes including the eggs, larval and transformation stages is important for gaining a better understanding of which species are spawning where and when, their hatching and nursery grounds, and ontogenetic habitat shifts during their development (Moftah et al. 2011; Ko et al. 2013). Traditionally, the identification of early-stage fishes to species level has used morphological characters, such as the body shape, pigmentation, meristic counts and measurements (Ko et al. 2013). Morphological identification can, however, be problematic as during the early life history stages many species in the same genus and even the same family share similar morphology (Ko et al. 2013). In addition, some features such as pigment, fins and scales are not fully developed, so there are not enough characters to identify to species level in some cases. Morphological identification of the early life history stages is especially difficult for rare and cryptic species. Morphologically cryptic fish species show ontogenetic colour variation and colour pattern similarities among different species and are difficult to identify as adults (Hubert et al. 2012). For these species there are almost no identification guides available for the larvae and early life history stages (Hubert et al. 2012).

The morphological characteristics of the early stages of fishes also change quickly and significantly through development: from the preflexion stage (when the notochord is still straight and caudal fin structures are just beginning to form) to the flexion stage (initial development of the caudal fin and supporting elements), to the postflexion stage (which begins after the completion of notochord flexion and ends at the onset of metamorphosis) to the transformation stage (start of metamorphosis to completion of fin-ray development and start of squamation) and finally to the juvenile stage (when attainment of full external meristics is complete) where individuals look more like small adults (Kendall 1981; Ko et al. 2013). As such, using traditional morphological techniques for identification to species level, particularly pertaining to the preflexion to transformation stages, can lead to misidentifications. To further compound this issue, taxonomists have different capabilities and skills in their own identification of specimens, which makes data comparison problematic (Ko et al. 2013). Given

these limitations the use of DNA barcoding for the identification of larvae and juveniles is a promising avenue (Mabragaña et al. 2011; Zhang 2011; Azmir et al. 2017).

DNA-based identification techniques have been developed and proven to be effective and powerful for fish identification (Ko et al. 2013; Zhang 2011; Azmir et al. 2017) and have been widely supported to identify species and uncover biological diversity (e.g. Hebert et al. 2003; Hubert et al. 2008; Zhang 2011; Moftah et al. 2011; Hubert et al. 2012). DNA barcoding is a technique designed to provide rapid and accurate species identifications, where a fragment of the cytochrome *c* oxidase subunit I (COI) mitochondrial gene region is used as a universal genetic "barcode" (Hebert et al. 2003; Hubert et al. 2008, 2012; Moftah et al. 2011; Ko et al. 2013). Cytochrome *c* oxidase is a mitochondrial protein, found in the inner mitochondrial membrane, and is an essential enzyme in the electron transport chain (Ward et al. 2009; Strüder-Kypke and Lynn 2010), which plays a central role in the metabolism of organisms (Hebert et al. 2003). It consists of several subunits, and the catalytic cytochrome *c* oxidase subunit I is encoded in the mitochondrial genome (Hebert et al. 2003; Strüder-Kypke and Lynn 2010). As such, the sequencing of this short standardised region of DNA allows for the identification of all life stages (Hebert et al. 2003).

While this approach has recently been used particularly for larval identification internationally (e.g. Becker et al. 2015; Ko et al. 2013; Victor et al. 2009) and in South Africa (e.g. Connell 2012; Steinke et al. 2016), many researchers when identifying fish larvae still rely on morphological identification in the laboratory (e.g. Leis 2008; Strydom 2008; Pattrick 2013; Strydom et al. 2015). Although, there are taxonomic guides available for identifying South Africa's adult coastal fish fauna (e.g. Smith and Heemstra 2003; Heemstra and Heemstra 2004), taxonomic guides for the early developmental stages of the full suite of South African coastal fishes are unavailable.

The aim of this chapter was to use DNA barcoding to correctly identify the early-life stages of species caught in the marine nearshore environment of Algoa Bay and the Swartkops and Sundays estuaries (details of study area are in Chapter Two), and as such to provide both morphological descriptions and images of postflexion and transformation stages for species which are not available in the literature.

3.2 Material and methods

3.2.1 Fish collection

Demersal fishes were collected using a 1.5 m wide conical, shoeless beam trawl net with a mouth height of 0.5 m; the body of the net was constructed from a 14 mm mesh and was 3 m long, tapering to a width of 0.5 m; a 1 m long x 0.5 m wide cod end of 6 mm mesh attached to the end of the net (Harrison et al. 2017). The lightweight, modified beam trawl net is highly efficient at capturing various life-stages of fish (7 mm cod end) (Nodo et al. 2017, 2018). In the present study, a smaller mesh size (6 mm cod end) was succesfully used to target early life-stages (post-flexion larvae and settlement stages).

At each sampling site the net was trawled for three minutes at a constant towing speed of approximately 2 knots. In the marine nearshore of Algoa Bay, the net was towed 50 m behind the boat and three replicates (approximately 200 m^2 apart) were done at each site, covering a total area of approximately 600 m^2. In the estuaries, the net was towed 20 m behind the boat and each site was trawled only once, covering approximately 200 m. Three replicates were done in the marine environment to cover a greater surface area (owing to the fact that the marine environment has much greater surface area compared to the estuaries) and one replicate was done in the estuarine environment as the estuaries are narrow, with a smaller surface area than the adjacent marine nearshore. Fish were identified and measured to the nearest millimetre total length (TL) and then released alive. Specimens, including larvae and small juveniles, that could not be identified in the field were anesthetized and then stored whole individually in Eppendorf tubes with 99% ethanol and were later measured (mm TL) and identified using DNA Barcoding in the laboratory. Larger individuals (early juveniles > 100 mm TL) that could not be identified in the field, were measured using a measuring board and Vernier caliper, photographed (using an iPhone 6s; 12 MP camera) and a fin clip (5 mm) taken from the pectoral fin and stored in an Eppendorf tube with 99% ethanol. These specimens were then released alive and no individuals were kept except for the finclip. When several (> 10 individuals) larger individuals of the same species were caught in the field, only one individual was randomly selected for DNA barcoding. All larger individuls were released alive. For specimens > 8 mm and < 100 mm, a clip cut was done in the laboratory. At the South African Environmental Observation Network (SAEON) Elwandle Coastal Node Microscope Laboratory, a dissecting microscope

(Zeiss Stemi 508) was used to take photographs (ZEISS-ZEN Imaging software) and measurements (mm TL) of all collected individuals that were stored whole.
All the sampling standards and laboratory procedures followed were approved by the Rhodes University Animal Research Ethics Committee (RU-AREC), #2019-1127-2140).

DNA barcoding

All genetic analyses were carried out at the South African Institute for Aquatic Biodiversity (SAIAB) Molecular Laboratory. DNA was extracted from each specimen using the "salting-out" technique (Sunnucks and Hales 1996) using either the fin clip sample, clip cut or in the case of very small larvae (preflexion larvae or < 8 mm TL) the whole specimen. A NanoDrop 1000 spectrophotometer (Thermo Scientific, USA) was used to measure the concentration and quality of the DNA extracts. A fragment of the mitochondrial cytochrome *c* oxidase subunit 1 gene (*COI*) was amplified using polymerase chain reaction (PCR). The primers dgHCO2198 (10 µM) and dgLCO1490 (10 µM) (Meyer 2003) were used to amplify samples. Each reaction included a total volume of 20 µl, with the following constituents: 10 µl of ReadyMix Taq (KapaTaq: Kapa Biosystems, South Africa); 4 µl of molecular graded water; 0.5 µl of each of the dgHCO2198 and dgLCO149 primers (10 µM); and 5 µl of DNA template. The PCR thermal cycling included an initial denaturing at 95 °C for 2 minutes, followed by 35 cycles of denaturing at 95 °C for 1 minute, annealing at 48 °C for 1 minute and an extension at 72 °C for 1 minute, followed by the final extension at 72 °C for 7 minutes. A negative control (without DNA) was used to rule out contamination of the amplifications. PCR products were then electrophoresed in a 1% agarose gel, which was stained with ethidium bromide (0.05 µg/ml) and observed under ultraviolet (UV) light to verify the amplification of the PCR product and to check for contamination. A 100 bp molecular ladder was used to verify the size of the amplification band.

The presence of a band (600–1000 bp) in individual samples and no bands in the negative control indicated a successful amplification. The amplified samples were then purified using an Exonuclease 1-Shrimp Alkaline Phosphatase (ExoSap10; Thermofisher Scientific, USA) protocol (Werle et al. 1994). Purified samples were sequenced using the BigDye v3.1 Cycle Sequencing Kit (Applied Biosystems, USA). The products were then precipitated using an ethanol-EDTA precipitation method (Sambrook and Russell 2001) to remove unincorporated dye-labelled terminators. After precipitation, the products were re-suspended in Hi-Di™ Formamide (Applied Biosystems, USA) and analysed on an ABI-Hitachi Genetic Analyser

3500 (Applied Biosystems, USA). Sequences were observed in Chromas Lite v2.1 (Technylesium Pty, Ltd) to verify the quality of the sequences. The Barcode of Life Data System (BOLD) (https://www.boldsystems.org/) (Ratnasingham and Hebert 2007) was then used for the comparison of sequences and species identification. Using BOLD, specimens that showed a sequence match of 99% or higher were assigned a species-level identification.

3.2.2 Species classification

Using morphology and biometrics (larval stages, length at one year, length at 50% maturity) for each species identified, individuals were categorized into five life-stage stages: postflexion stage, transformation stage, Young-of-the-Year (YOY) juveniles, late juveniles and adults (maturity stages) (Table 1).

Table 3.1: Description of different life stages of fish species (adapted from https://access.afsc.noaa.gov/ichthyo/StageDefPage.php)(Kendall 1984)

Life Stage	Description of the life stages
Postflexion larvae	The postflexion stage begins after the completion of notochord flexion and ends at the onset of metamorphosis (transformation).
Transformation stage	The start of metamorphosis to completion of fin-ray development as well as scale formation. Changes in body shape and pigment pattern which include fin migration, photophore formation, loss of specialized larval characters, eye migration (in some) and scale formation.
Settlement stage	The transition from the pelagic to the benthic environment
Young-of-the-Year (YOY) juveniles	The completion of fin-ray development and complete ossification of the skeleton occurs during this period. Juveniles are up to one year old (length at one year).
Late juveniles	This stage ends with the attainment of sexual maturity (length at 50% maturity).
Adults	The stage begins after fish have attained sexual maturity (length-at-50%-maturity)

3.3 Results and discussion

3.3.3 Species identification by DNA barcoding

One hundred specimens (random outcome number from the sample) were collected for DNA barcoding, of which 86 specimens were positively identified to species level using CO1 sequences, while 14 failed to amplify by PCR and were only identified morphologically (Table 3.2). Most of the specimens were transformation stage (n = 31), followed by postflexion stage (n = 28), and young-of-the-year (YOY) juveniles (n = 27). Eighteen species were positively identified using DNA barcoding (Table 3.2). The Sparidae family contributed the greatest number of species (n = 5), followed by Sciaenidae (n = 4) (Table 3. 2). Almost all of the Sparidae positively identified were in the transformation stage. The highest number (n = 26) of positively identified specimens were from the Haemulid, *Pomadasys olivaceus*, of which most were also in the transformation stage. These species were dominated by marine estuarine-opportunist (MED) species, which were caught in all the localities (Algoa Bay, Sundays Estuary and Swartkops Estuary), followed by marine species (M) recorded only in the Algoa Bay marine nearshore (Table 3.2).

Table 3.2: Specimens collected and positively identified using DNA barcoding in Algoa Bay (A), Sundays Estuary (S) and Swartkops Estuary (SW) during the sampling period (July 2017 – September 2019). The number of individuals for each species per length range, life stage, fish guild and locality.

Family	Species	Number of individuals	Length (range: mm TL)	Life stage	Fish guild	Locality
Sparidae	*Rhabdosargus holubi*	2	19-23	Transformation	MED	SW,A
	Rhabdosargus globiceps	3	20-22	Transformation	MEO	A,S
	Rhabdosargus thorpei	1	23	Transformation	MEO	S
	Sarpa salpa	1	31	YOY juvenile	MEO	A
	Spondyliosoma emarginatum	6	21-30	Transformation	MEO	A,S,SW
		2	40-45	YOY juvenile		A,S
Haemulidae	*Pomadasys olivaceus*	9	14-17	Larvae	MEO	A,S,SW
		13	20-30	Transformation		A,S,SW
		3	40-90	YOY juvenile		A,S,SW
	Pomadasys commersonnii	4	30-60	YOY juvenile	MED	S
Sciaenidae	*Argyrosomus japonicus*	1	28	Transformation	MED	S
		2	34-47	YOY juvenile		S
	Argyrosomus inodorus	8	8-17	Larvae	MS	A
		2	20-22	Transformation		A
		5	76-147	YOY juvenile		A
	Umbrina canariensis	2	15-23	Transformation	M	A
		3	30-86	YOY juvenile		A
	Atractoscion aequidens	5	15-17	Larvae	M	A
Gobiidae	*Psammogobius knysnaensis*	1	23	YOY juvenile	E&M	SW
	Caffrogobius saldanha	3	12-16	Larvae	E&M	A,SW
	Glossogobius callidus	1	20	Transformation	E&F	S
Engraulidae	*Engraulis capensis*	3	21-30	Larvae	M	A
Carangidae	*Trachurus trachurus*	2	28-40	YOY juvenile	M	A
Triglidae	*Chelidonichthys kumu*	2	27-95	Juvenile	MS	A
Blenniidae	*Omobranchus woodi*	2	20-49	Juvenile	E&M	SW

Fish guild from Whitfield (2019): E&M = estuarine and marine species, E&F = estuarine and freshwater, MEO = marine estuarine-opportunist species, MED = marine estuarine-dependent species, MS = marine stragglers.

Sparidae

The five Sparidae species positively identified through DNA barcoding included three *Rhabdosargus* species (*R. holubi*, *R. thorpei*, and *R. globiceps*), *Spondyliosoma emarginatum* and *Sarpa salpa* (Table 3.2). The *Rhabdosargus* species barcoded; *R. holubi* (19 and 23 mm TL), *R. thorpei* (23 mm TL) and *R. globiceps* (20, 21 and 22 mm TL), were all transformation stage and could not be differentiated morphologically. Although all had indistinct vertical bars with a slightly compressed body and were moderate to deep in shape, the bars were more pronounced in *R. globiceps* (Figure 3.1, Table 3.2). *Rhabdosargus holubi* and *R. thorpei* could also be distinguished by their short snout and big eyes, while *R. globiceps* has a pointy and longer snout. This study provides the first description of distinguishing characters that can be used to morphologically describe the transformation stages of these three Sparidae species as there are currently no guides available for this stage (Figure 3.1).

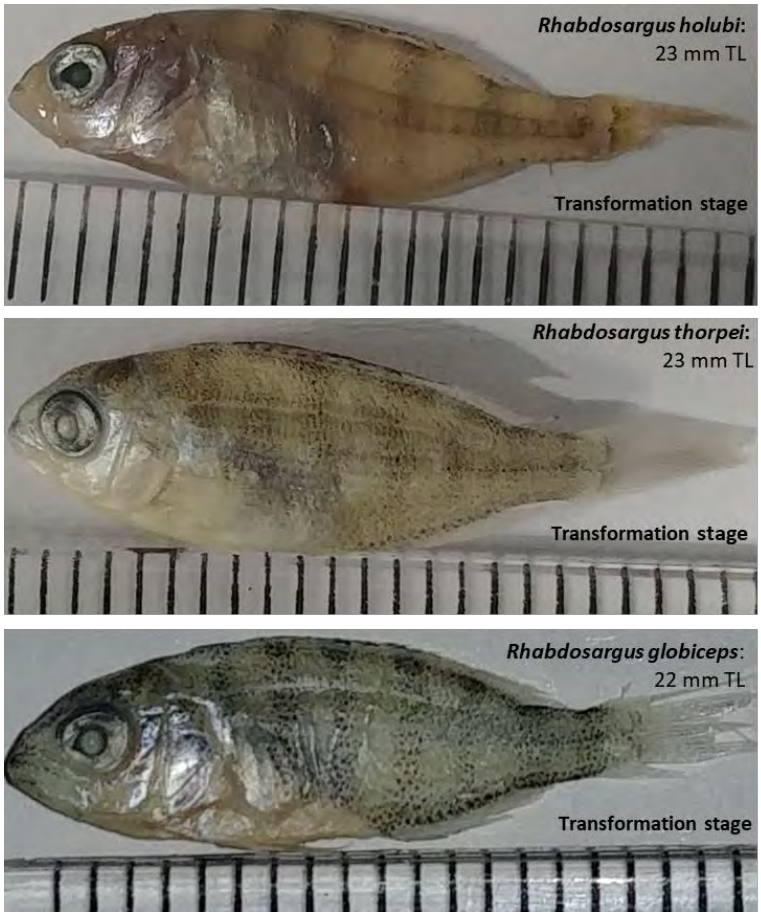

Figure 3.1: *Rhabdosargus* spp (Sparidae) transformation stage individuals caught in the marine nearshore environment and Sundays and Swartkops estuaries between July 2017 and September 2019.

One transformation stage *Sarpa salpa* (31 mm TL) was caught during this study and morphologically this species was similar to the transformation stage *Spondyliosoma emarginatum* (Figure 3.2). *Sarpa salpa* is highly pigmented with small black pigment spots on the dorsal surface with elongated body, while, *S. emarginatum* has black spots only on the ventral surface near the anal fin to the caudal peduncle (Figure 3.2).

Figure 3.2: Transformation stage *Sarpa salpa* and *Spondyliosoma emarginatum* caught in the marine nearshore environment and Sundays Estuary during the sampling period (July 2017 – September 2019).

Postflexion and transformation stage *S. emarginatum* were first morphologically identified as *Pomadasys olivaceus* (Haemulidae) based on their short and compressed body shape, and similar pigmentation (Figure 3.3). After positive identification with DNA barcoding, it was then noted that transformation stage *S. emarginatum* are far more heavily pigmented than *P. olivaceus* over nearly the entire head, with pigmentation patterns changing markedly during development. Literature indicates that preflexion *S. emarginatum* larvae have little pigment on the ventral surface near the gut, while postflexion larvae are characterized by visible medio-lateral pigmentation (Beckley 1989), which makes them easily distinguishable from similar stages of larvae of other sparids (Beckley 1989). Although the larvae of these species have been described, relatively little is known about the transformation stages. Therefore, the present study provides the first description/distinguishing characters that can be used to morphologically identify transformation stage *S. emarginatum* in the field.

Figure 3.3: Flexion, postflexion, transformation and young-of-the-year a) *Spondyliosoma emarginatum* (Sparidae) and b) *Pomadasys olivaceus* (Haemulidae) caught in the marine nearshore and Sundays Estuary during the sampling period (July 2017 – September 2019).

Haemulidae

Pomadasys olivaceus and *P. commersonnii* are also very similar in appearance during their larval and transformation stages (Figure 3.4). The distinction between the transformation stages lies in their body shape, which is elongated with a pointy snout in *P. commersonnii*, while *P. olivaceus* has a short snout and roundish body and the juveniles usually have a black spot on the operculum (Figure 3.4). *Pomadasys commersonni* transformation stages are more highly

pigmented on the dorsal and ventral surface than *P. olivaceus*. Most specimens identified with DNA barcoding were *P. olivaceus*. There are currently no guides describing the transformation stages of both species. *Pomadasys commersonni* become darker on the dorsal surface as they increase in size/length (Figure 3.4). *Pomadasys commersonnii* adults and juveniles have pectoral fins which equal their head length and are longer than their pelvic fins, while those of the *Pomadasys olivaceus* are shorter compared to *Pomadasys commersonnii* (Heemstra and Heemstra 2004) and this can be clearly seen in > 50 mm TL individuals.

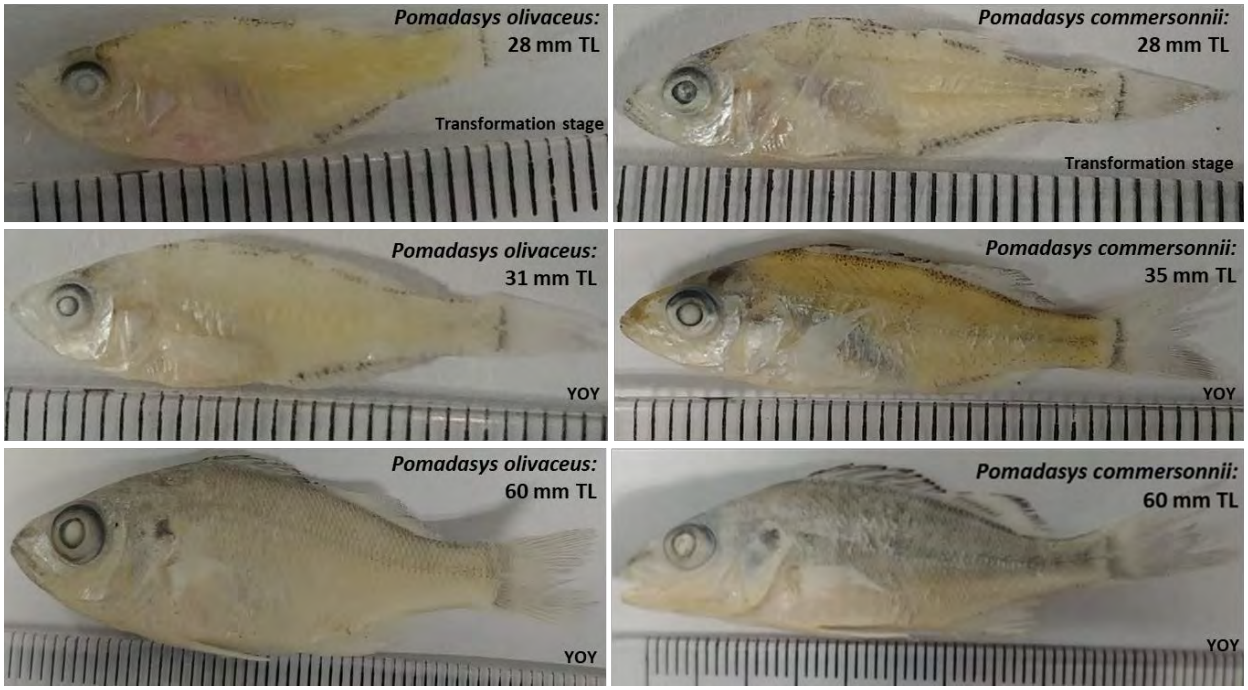

Figure 3.4: Transformation stage and young-of-the-year juvenile *Pomadasys* species (Haemulidae) caught in the marine nearshore environment and the Sundays Estuary during the sampling period (July 2017 – September 2019).

Sciaenidae

Four sciaenid species were identified by DNA barcoding namely, *Argyrosomus japonicus* (28 – 47 mm TL), *Argyrosomus inodorus* (8 – 154 mm TL), *Umbrina canariensis* (14 – 83 mm TL), and *Atractoscion aequidens* (15 mm TL) (Figure 3.5 & 3.6, Table 3.2). All stages of the closely related *A. japonicus* and *A. inodorus* are morphologically very similar (Figure 3.6). The differences between the transformation stages of the two species (morphologically) is that *A. japonicus* has black dots on the ventral surface from the anal fin to the caudal peduncle and *A. inodorus* is only pigmented (black dots) near the anal fin (Figure 3.5 & 3.6). *Argyrosomus inodorus* postflexion and transformation stages have larger eyes than *A. japonicus*. Large

individuals including adults of *A. japonicus* have a shorter deeper peduncle, shorter pectoral fin (tip of the fin not reaching the vertical at the tip of folded pelvic fin) and they also attain a larger size than *A. inodorus* (Heemstra and Heemstra 2004). A full description of the morphological characteristics of adult *A. japonicus* is provided by Griffiths and Heemstra (1995). In addition, the description of larval development of *A. hololepidotus* by Beckley (1990) collected in the marine nearshore region of Algoa Bay could be that of *A. inodorus* (Beckley 1990). Connell (2012) also gave a description of early-life stages (eggs to juveniles) of some marine species including *A. japonicus* and *A. aequidens* collected from the east coast of South Africa. The larval and juvenile description provided by Connell (2012) is similar to the life stages recorded during the sampling period. For example, *A. aequidens* (15 mm TL) is similar in appearance with the specimen *A. aequidens* (9.0 mm SL) Connell (2012).

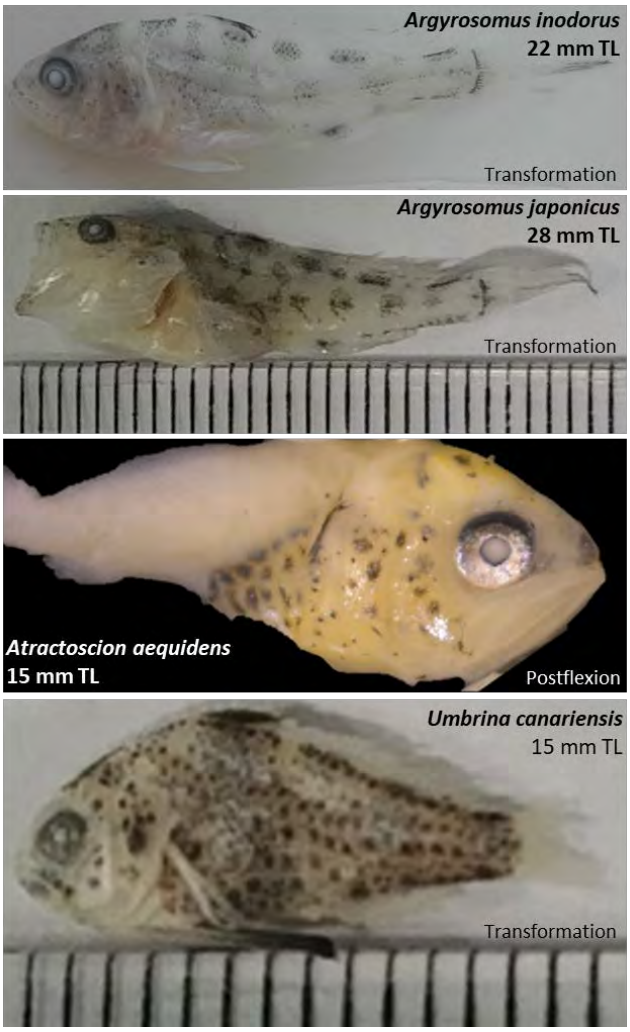

Figure 3.5: Postflexion and transformation stages of Sciaenidae species caught in the marine nearshore environment during the sampling period (July 2017 – September 2019).

Figure 3.6: Postflexion, transformation stage and young-of-the-year juveniles of *Argyrosomus inodorus* and *Argyrosomus japonicus* (Sciaenidae) caught in the marine nearshore environment and Sundays Estuary during the sampling period (July 2017 – September 2019).

Atractoscion aequidens and *U. canariensis* (Figure 3.5 & 3.7) were identified first in the laboratory using morphological characteristics and then confirmed using DNA barcoding. *Umbrina canariensis* and *A. aequidens* are known to occur in Algoa Bay (Smale 1985; Beckley 1990). The larvae of *U. canariensis* (Figure 3.5 & 3.7) can be distinguished from those of *Argyrosomus spp.* by a prominent dorso-ventral pigment band across the body, pigmented pelvic fins and smaller size at flexion (Beckley 1990). Although there is little information available on the larval stages of *A. aequidens,* Beckley (1990) mentioned that *A. aequidens* differs from other *Argyrosomus spp*. by having higher dorsal and anal fin ray counts, which would allow late postflexion larvae of the two species to be distinguished.

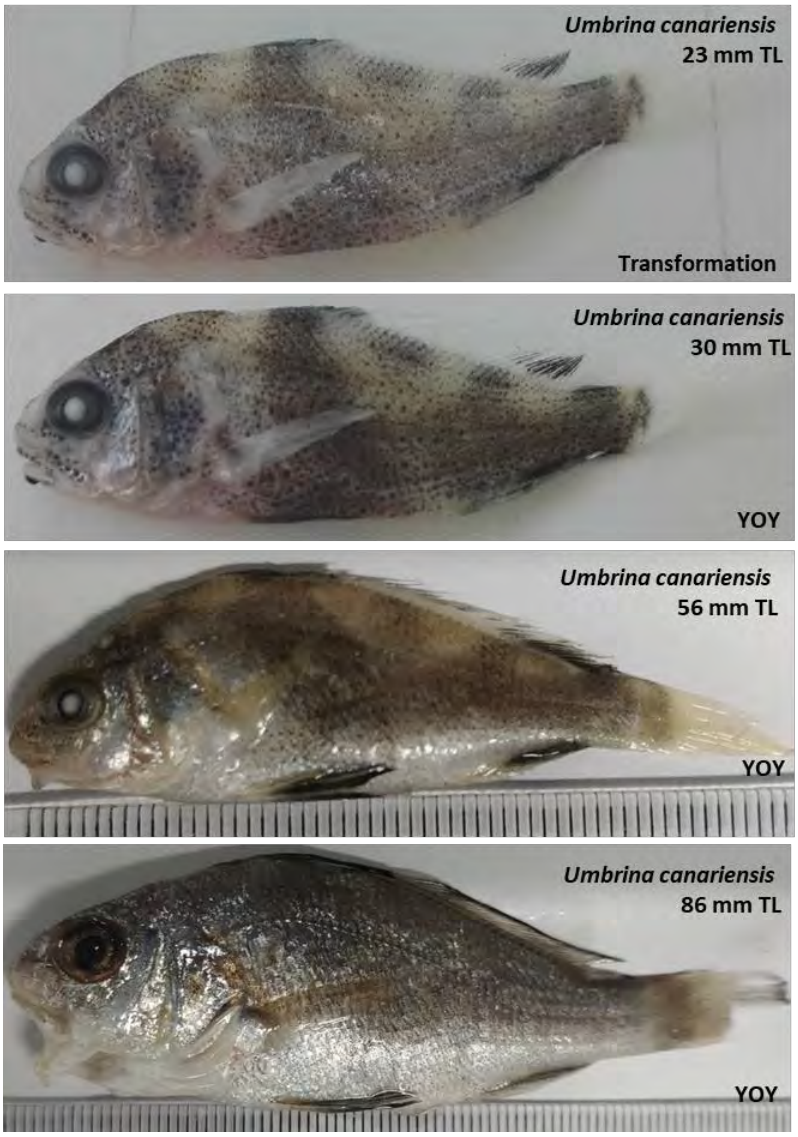

Figure 3.7: Transformation and young-of-the-year juveniles of *Umbrina canariensis* (Sciaenidae) caught in the marine nearshore environment during the sampling period (July 2017 – September 2019).

Gobiidae

Gobiidae species including *Caffrogobius saldanha* (12 – 15 mm TL), *Psammogobius knysnaensis* (17 mm TL*)* and *Glossogobius callidus* (21 mm TL) were also identified by DNA barcoding (Figure 3.8; Table 3.2). All specimens collected were transformation stages. Owing to the similarities between gobies during the larval and transformation stages, *C. saldanha* were misidentified morphologically as *Caffrogobius nudiceps* in the laboratory. The prominent gas bladder and pigminant patterns on the ventral side of the body were some of the features that were responsible for the misidentification in the lab. *Caffrogobius saldanha* was mostly caught in the shallow marine nearshore environment, usually on sand and muddy bottoms. *Caffrogobius saldanha* is an estuarine and marine species known to occur in estuarine, intertidal and inshore waters, usually over mud areas (Goren 1996).

Figure 3.8: Gobiidae species caught in the marine nearshore environment, Sundays and Swartkops estuaries during the sampling period (July 2017 – September 2019).

Other species

Other species not easily identified morphologically in the field, which were barcoded successfully included single individuals of postflexion *Engraulis capensis* (Engraulidae), YOY juvenile *Trachurus trachurus* (Carangidae), YOY juvenile *Chelidonichthys kumu* (Triglidae)

and YOY juvenile *Omobranchus woodi* (Blenniidae) (Figure 3.9; Table 3.1). *Diplodus capensis* was identified morphologically but the PCR failed (Table 3.3).

Figure 3.9: Postflexion and young-of-the-year juveniles of the species from the families Engraulidae, Carangidae, Triglidae and Blenniidae caught in the marine nearshore environment and Swartkops Estuary during the sampling period (July 2017 – September 2019).

Table 3.3: Individuals that were identified morphologically and then positively identified using DNA barcoding, including those samples that failed PCR. Specimens were recorded in the marine nearshore environment of Algoa Bay, and in the Sundays and Swartkops estuaries during the sampling period (July 2017 – September 2019).

Morphological identified	Locality	Life stage	Length (mm TL)	DNA results
Argyrosomus japonicus	Algoa Bay	Larvae	14	*Argyrosomus inodorus*
Argyrosomus japonicus	Algoa Bay	Larvae	15	*Argyrosomus inodorus*
Argyrosomus japonicus	Algoa Bay	Settlement	24	*Argyrosomus inodorus*
Caffrogobius nudiceps	Algoa Bay	Larvae	12	*Caffrogobius saldanha*
Caffrogobius nudiceps	Swartkops	Larvae	16	*Caffrogobius saldanha*
Caffrogobius nudiceps	Sundays	Larvae	16	*Caffrogobius saldanha*
Pomadasys olivaceus	Sundays	Juvenile	30	*Pomadasys commersonnii*
Spondyliosoma emarginatum	Algoa Bay	Larvae	16	*Pomadasys olivaceus*
Pomadasys commersonnii	Sundays	Larvae	17	*Pomadasys olivaceus*
Spondyliosoma emarginatum	Algoa Bay	Larvae	17	*Pomadasys olivaceus*
Pomadasys commersonnii	Sundays	Larvae	18	*Pomadasys olivaceus*
Pomadasys commersonnii	Sundays	Larvae	19	*Pomadasys olivaceus*
Pomadasys commersonnii	Sundays	Settlement	23	*Pomadasys olivaceus*
Pomadasys commersonnii	Sundays	Settlement	24	*Pomadasys olivaceus*
Pomadasys commersonnii	Sundays	Settlement	25	*Pomadasys olivaceus*
Pomadasys commersonnii	Sundays	Settlement	28	*Pomadasys olivaceus*
Pomadasys commersonnii	Sundays	Settlement	30	*Pomadasys olivaceus*
Spondyliosoma emarginatum	Algoa Bay	Juvenile	30	*Pomadasys olivaceus*
Spondyliosoma emarginatum	Algoa Bay	Settlement	33	*Pomadasys olivaceus*
Spondyliosoma emarginatum	Algoa Bay	Settlement	33	*Pomadasys olivaceus*
Spondyliosoma emarginatum	Algoa Bay	Juvenile	35	*Pomadasys olivaceus*
Pomadasys commersonnii	Sundays	Settlement	36	*Pomadasys olivaceus*
Spondyliosoma emarginatum	Sundays	Juvenile	42	*Pomadasys olivaceus*
Glossogobius callidus	Swartkops	Juvenile	23	*Psammogobius knysnaensis*
Lithognathus lithognathus	Sundays	Settlement	20	*Rhabdosargus globiceps*
Lithognathus lithognathus	Swartkops	Settlement	20	*Rhabdosargus globiceps*
Lithognathus lithognathus	Algoa Bay	Settlement	22	*Rhabdosargus globiceps*
Lithognathus lithognathus	Algoa Bay	Settlement	19	*Rhabdosargus holubi*
Lithognathus lithognathus	Swartkops	Settlement	23	*Rhabdosargus holubi*
Lithognathus lithognathus	Sundays	Settlement	23	*Rhabdosargus thorpei*
Pomadasys olivaceus	Algoa Bay	Juvenile	36	*Sarpa salpa*
Pomadasys olivaceus	Algoa Bay	Settlement	23	*Spondyliosoma emarginatum*
Pomadasys olivaceus	Swartkops	Settlement	23	*Spondyliosoma emarginatum*
Pomadasys olivaceus	Sundays	Settlement	25	*Spondyliosoma emarginatum*
Pomadasys olivaceus	Algoa Bay	Settlement	25	*Spondyliosoma emarginatum*
Pomadasys olivaceus	Sundays	Settlement	27	*Spondyliosoma emarginatum*
Pomadasys olivaceus	Algoa Bay	Juvenile	35	*Spondyliosoma emarginatum*
Pomadasys olivaceus	Sundays	Juvenile	36	*Spondyliosoma emarginatum*
Pomadasys olivaceus	Algoa Bay	Juvenile	40	*Spondyliosoma emarginatum*
Pomadasys olivaceus	Algoa Bay	Juvenile	45	*Spondyliosoma emarginatum*
Umbrina robinsoni	Algoa Bay	Settlement	17	*Umbrina canariensis*
Umbrina robinsoni	Algoa Bay	Settlement	18	*Umbrina canariensis*
Pomadasys commersonnii	Algoa Bay	Settlement	30	*Pomadasys olivaceus*
Pomadasys commersonnii	Algoa Bay	Settlement	30	*Pomadasys olivaceus*
Pomadasys commersonnii	Algoa Bay	Settlement	33	*Pomadasys olivaceus*
Pomadasys commersonnii	Algoa Bay	Juvenile	34	*Pomadasys olivaceus*
Pomadasys commersonnii	Algoa Bay	Juvenile	34	*Pomadasys olivaceus*
Parablennius cornutus	Algoa Bay	Juvenile	46	Failed PCR
Sardinops sagax	Algoa Bay	Larvae	30	Failed PCR
Sardinops sagax	Algoa Bay	Larvae	31	Failed PCR
Sardinops sagax	Algoa Bay	Larvae	30	Failed PCR

Diplodus capensis	Algoa Bay	Larvae	10	Failed PCR
Diplodus capensis	Algoa Bay	Larvae	17	Failed PCR
Pomadasys olivaceus	Algoa Bay	Juvenile	112	Failed PCR
Pomadasys olivaceus	Algoa Bay	Juvenile	50	Failed PCR
Pomadasys commersonnii	Sundays	Juvenile	60	Failed PCR
Argyrosomus inodorus	Algoa Bay	Juvenile	154	Failed PCR
Argyrosomus inodorus	Algoa Bay	Juvenile	140	Failed PCR
Argyrosomus inodorus	Algoa Bay	Juvenile	135	Failed PCR
Umbrina canariensis	Algoa Bay	Settlement	25	Failed PCR
Umbrina canariensis	Algoa Bay	Settlement	24	Failed PCR
Umbrina canariensis	Algoa Bay	Settlement	25	Failed PCR

3.4 Conclusion

It was evident from the results of the DNA barcoding that not all of our field identifications using morphological techniques were correct, with a total of approximately 47 specimens representing ten species identified incorrectly. Field identification was particularly challenging in cryptic species such as *A. inodorus* and *A. japonicus,* due to similarities in body shapes and pigment patterns among species. DNA barcoding has been used successfully to aid in the identification of species that are not easily identified in the field, particularly those with morphological similarities, as recent studies have shown that pigment patterns change with life stage and different habitats, thus complicating field identification (e.g. Becker et al. 2015; Ko et al. 2013; Victor et al. 2009).

DNA barcoding has the advantage of being a reliable, effective and available tool in the identification of larval species (Ko et al. 2013). It also requires significantly less training than morphological identification. In order to ensure the accurate identification of larval and settlement stage fishes, taxonomic studies should use a combination of both morphology and DNA barcoding methods (Azmir et al. 2017). Some researchers have recommended the use of both morphological and DNA barcoding for the identification of larval and juvenile fishes (Hulley et al. 2018; Ko et al. 2013). As such, this study revealed that positive and successful identification of larval and transformation stages of fish to species level was only possible when DNA barcoding was used, and has subsequently allowed for detailed images showing the morphology and pigmentation patterns of several demersal larval and transformation stage marine fish species, which were previously unavailable.

CHAPTER FOUR

The demersal fish assemblage of the shallow marine nearshore and estuarine waters of Algoa Bay and relationships with environmental parameters

4.1 Introduction

Estuaries can have several ecotones, which are defined as a gradient or transition zone between ecosystems (Attrill and Rundle 2002; Elliot and Whitfield 2011). These ecotones include the transition between freshwater and estuarine systems at the head of the estuary and the transition between estuarine and marine systems at the mouth of the estuary, which can extend into the marine nearshore. The extent and locality of these ecotones varies with estuary type and position (Elliot and Whitfield 2011). In the northern Hemisphere, where estuarine systems are often much larger than those in the southern Hemisphere, the extent of both ecotones (at the head and mouth) are often much greater than in the southern Hemisphere (Elliot and Whitfield 2011). For example, Martino and Able (2003) sampled fish across a gradient of sites (over 40 km long) from the upper reaches of the Mullica Estuary, Great Bay to the inner continental shelf in southern New Jersey (USA) and found that the estuarine/marine ecotone extended into the bay. Globally, relatively few studies have focussed on fish community change across the entire freshwater to marine seascape (e.g. Potter et al. 2000).

The relatively low number of studies which have simultaneously sampled both estuarine and nearshore marine environments has been attributed to the challenge of sampling both environments with the same sampling gear at the same time (Woodland et al. 2012). Trawl nets (such as beam trawls) overcome some of these sampling limitations and are effective at catching a variety of soft bottom demersal species and size classes of juvenile and adult life stages in both estuarine and marine nearshore environments (e.g. Martino and Able 2003; Able et al. 2006; Woodland et al. 2012; Harrison et al. 2017). Despite the effectiveness of this gear in sampling across the freshwater to marine gradient, no studies in South Africa have simultaneously sampled soft-bottom habitats in estuaries and the marine nearshore concurrently.

Several environmental variables have been shown to affect the demersal fish assemblages within estuaries (e.g. Vasconcelos et al. 2013). For example, in South Africa, studies using

trawl nets as fishing gear have shown that freshwater inflow, salinity and sediment type affect the demersal fish assemblage throughout the estuary as well as the size and temporal presence of ecotones within estuaries (e.g. Richardson et al. 2006; Vorwerk et al. 2008; Nodo et al. 2017, 2018). Some benthic fish species are associated with the sandier, marine-dominatedlower reaches, while others are associated with the muddier middle and upper reaches of the estuary (e.g. Richardson et al. 2006; Bailey and James 2013). Distribution patterns of demersal fish assemblages also differ among different types of estuaries. In the temperate freshwater-deprived and marine-dominated Kariega Estuary (often with the reversed salinity gradient), South Africa, there is typically no freshwater/estuarine ecotone and demersal fish assemblages only differ between the mouth region and the rest of the estuary (Richardson et al. 2006; Bailey and James 2013). Following an extreme flood event in the system and the re-establishment of an estuarine salinity gradient, the freshwater/estuarine ecotone was temporarily restored (Nodo et al. 2018). While in the nearby freshwater-enriched Great Fish Estuary, the freshwater/estuarine ecotone is extensive (extends from the head region of the estuary to the mouth) and the estuarine/marine ecotone is lost during flooding (Nodo et al. 2017).

In the marine nearshore environment of temperate South Africa, trawling studies on demersal fish assemblages have been undertaken off the mouth of the Swartkops Estuary, Algoa Bay, in water between 5 and 7 m deep (Beckley 1984) and extensively during the summer of 1980 from Mossel Bay to Algoa Bay in water from 4.5 to 100 m deep (Buxton et al. 1984; Smale 1984; Wallace et al. 1984a). Beckley (1984) found that species caught off the Swartkops Estuary mouth were typical inshore marine species, similar to those caught further offshore (Wallace et al. 1984a). This led to Beckley (1984) concluding that the estuary had little influence on the fish composition caught off the estuary mouth, suggesting that the estuary mouth functions as a biogeographic boundary between two different estuarine and marine fish communities (i.e. the estuarine/marine ecotone does not extend into the nearshore marine environment). Trawling was not, however, done concurrently in the Swartkops Estuary to determine the demersal fish assemblage in the estuary. This research was also undertaken four decades ago and since then there has been no research on demersal fish communities of the marine nearshore environment of temperate South Africa, nor across the freshwater marine seascape.

The main aim of this chapter was to use data on demersal fish communities and physico-chemical parameters from soft-bottom benthic habitats in two permanently open estuaries

(along the freshwater to marine gradient) and the marine nearshore environment (5 – 10 m) of Algoa Bay, to (i) examine whether estuarine and marine nearshore environments have distinct fish assemblages, (ii) determine the physical drivers of demersal fish assemblages and (iii) assess the spatial extent of the freshwater/estuarine and estuarine/marine ecotones within two different estuary/coastal seascapes.

4.2 Materials and Methods

4.2.1 Sampling

Demersal fishes were sampled in the shallow (5-10 m depth) marine nearshore in Algoa Bay as well as in the Sundays and Swartkops estuaries for a total of twelve sampling trips between July 2017 and September 2019 (study site and sampling methods are described in detail in Chapter Two and Three). Physico-chemical characteristics were recorded at each sampling site using a YSI (6290) multi-parameter probe and these included water temperature (ºC), salinity, turbidity (NTU) and dissolved oxygen (mg/l). Sediment samples were also collected using a cone dredge for particle size and sediment organic content analysis. Sampling methods for physico-chemical characteristics are described in detail in Chapter Two.

Catch-per-unit-effort (CPUE) was used as an index of abundance, where fish catches were expressed as total number of individuals caught in each site divided by the area trawled (numbers per 1000 m^2) (Harrison et al. 2017); calculated using the formula below:

$$CPUE = \ total\ number\ of\ individuals\ at\ \ each\ site \div area\ trawled\ \times 1000$$

4.2.2 Data analysis

Environmental data

To investigate the relationship between demersal fish assemblages and environmental variables, the mean, median and range of salinity, turbidity (NTU), temperature (°C), dissolved oxygen (mg/l), organic content (%) and silt (%) were calculated for the bottom waters of each site.

Species assemblage

Using information provided in FishBase (Froese and Pauly 2011), all the fish captured using the shoeless beam trawl were grouped into either benthic species (B) that live on or in the bottom substrate, bentho-pelagic species (BP) that live near the bottom, and pelagic species (P) that are found in the water column.

Species were also categorised into estuary association categories using the classification scheme of Potter et al. (2015) applied to South African fish species (Whitfield 2019). Using this scheme, marine stragglers (MS) are described as species that spawn at sea and typically enter estuaries in only low numbers. Marine species (M) do not occur in estuaries and occur only in the marine environment. Marine estuarine-opportunist (MEO) species regularly enter estuaries in substantial numbers, particularly as juveniles but use estuaries to varying degrees, also using marine nearshore as alternative nursery areas. Marine estuarine-dependents (MED) are described as species whose juveniles require sheltered estuarine habitats as nursery areas and are not found in the marine environment during this stage. Solely estuarine species (SE) are found only in estuaries. Estuarine and marine species (E&M) are estuarine species that are also represented by marine populations. Estuarine and freshwater species (E&F) are estuarine species which are also represented by freshwater populations (Potter et al. 2015).

To test for the effect of changes in salinity, turbidity (NTU), dissolved oxygen (mg/l), organic content (%) and silt (%) on demersal fish assemblages, linkage tree analyses (LINKTREE in Primer) were conducted based on mean environmental variables (averaged per site) and CPUE (by site). For this analysis, fish abundance data were square root transformed prior to analysis, environmental variables were normalised and the Bray-Curtis (fish data) and Euclidean (environmental variables) similarity matrices were performed (Clarke et al. 2008). The LINKTREE procedure (a non-metric multivariate regression tree technique) uses a constrained clustering technique to construct a hierarchical tree (cluster group) to identify the subset of environmental variables thresholds that best explain significant patterns based on assemblage data (Clarke et al. 2008). The groupings were confirmed statistically through similarity profile routine (SIMPROF) tests at a significance level of 0.05, conducted with 999 permutations between the mean environmental variables and mean CPUE per site (Clarke et al. 2008). Non-metric multidimensional scaling (nMDS) ordinations were used to visualise the patterns identified in the linkage tree analyses as well as the contribution or abundance of species by guild, family and habitat association in each site. These analyses were performed in PRIMER statistical package version 7.0.13 (Clarke and Warwick 1994).

To visually assess the distribution of the most abundant species from the upper reaches of both estuaries to the marine nearshore sites, a shade plot was constructed based on the LINKTREE cluster analysis. These analyses were performed in PRIMER statistical package version 7.0.13 (Clarke and Warwick 1994).

To determine spatial and seasonal changes in species assemblages across the estuarine-marine salinity gradient in the two different estuaries, sites were divided into two demersal seascapes. The first being the Sundays freshwater to marine seascape, which was from the upper reaches of the Sundays Estuary (Site S9) to the adjacent marine nearshore sites (sites A7, A6 and A5) (Figure 4.1). The Swartkops freshwater to marine seascape was from the upper reaches of the Swartkops Estuary (Site SW7) to the adjacent marine nearshore sites (sites A4, A3, A2 and A1) (Figure 4.1).

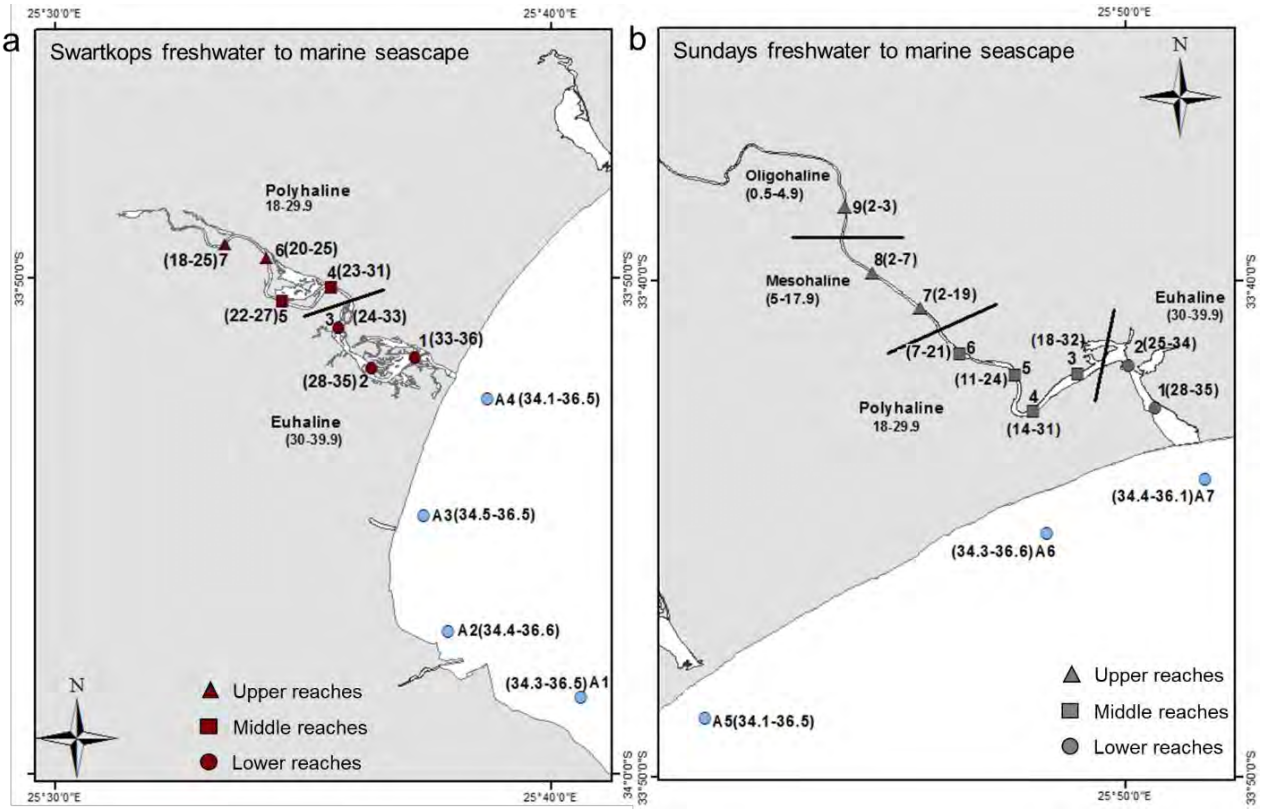

Figure 4.1: Map showing sampling sites in the a) Swartkops Estuary to Algoa Bay marine nearshore seascape and b) Sundays Estuary to Algoa Bay marine nearshore seascape. Salinity gradients from the upper reaches of the two estuaries to the Algoa Bay nearshore marine sites according to the Venice System, based on the mean salinity at the bottom of the water column during the study period (July 2017 – September 2019) are depicted.

Environmental data for each seascape were presented using boxplot graphs constructed in the R environment for computing statistics version 3.6.0 (R Development Core Team 2019). Spearman rank correlation coefficients were used to further explore significant relationships between environmental variables and the CPUE of the most abundant species in all three sampling localities (Sundays, Swartkops and Algoa Bay marine nearshore). Correlations were

considered significant at a level of $P < 0.05$. This analysis was conducted in the R environment for computing statistics version 3. 6. 0 (R Development Core Team 2019).

4.3 Results

4.3.1 Species composition

A total of 797 fishes (29 species) were collected in the marine nearshore and 6437 fishes (28 species) were caught in the estuarine environment (3752 and 2685 individuals, with 24 and 20 species recorded in the Sundays and Swartkops estuaries, respectively (Table 4.1). In the marine nearshore, the total catch from all the sites (mean of all sites) was dominated by *Galeichthys feliceps* (9.0 fish per 1000 m^2; 39%), *Pomadasys olivaceus* (5.2 fish per 1000 m^2; 23%), *Argyrosomus inodorus* (1.3 fish per 1000 m^2; 6%), *Cynoglossus zanzibarensis* (1.2 fish per 1000 m^2; 5%), *Dasyatis chrysonota* (1.0 fish per 1000 m^2; 4%) and *Umbrina canariensis* (0.7 fish per 1000 m^2; 3%) (Table 4.1).

In the Sundays Estuary, catches were dominated by *Solea turbynei* (33.3 fish per 1000 m^2; 18%), *Gilchristella aestuaria* (27.6 fish per 1000 m^2; 15%), *Caffrogobius gilchristi* (27.3 fish per 1000 m^2, 14%), *Rhabdosargus holubi* (24.9 fish per 1000 m^2; 13%), *Psammogobius knysnaensis* (21.3 fish per 1000 m^2; 11%) and *Glossogobius callidus* (18.5 fish per 1000 m^2; 10%) (Table 4.1). The total catch in the Swartkops Estuary was dominated by *Rhabdosargus holubi* (42.6 fish per 1000 m^2; 24%), *Caffrogobius gilchristi* (27.2 fish per 1000 m^2, 16%), *Heteromycteris capensis* (24.7 fish per 1000 m^2; 14%), *Glossogobius callidus* (24.7 fish per 1000 m^2; 14%), *Psammogobius knysnaensis* (24.2 fish per 1000 m^2; 14%) and *Solea turbynei* (17.2 fish per 1000 m^2; 10%) (Table 4.1).

Nine species were caught in both the estuarine and marine nearshore environments and these included *R. holubi*, *Diplodus capensis, Spondyliosoma emarginatum, Rhabdosargus globiceps, Caffrogobius saldanha, G. feliceps, Heteromycteris capensis, Solea turbynei* and *P. olivaceus* (Table 4.1). Twenty species were restricted to the marine nearshore environment, and included *Geneion honckenii*, *Atractoscion aequidens*, *A. inodorus*, *Caffrogobius agulhensis*, *Cheimerius nufar*, *Chelidonichthys kumu, Cynoglossus capensis, C. zanzibarensis, D. chrysonota*, *Engraulis capensis*, *Gymnura natalensis*, *Myliobatis aquila*, *Parablennius cornutus, Raja clavata*, *Raja miraletus*, *Rhinobatos annulatus, Sardinops sagax, Sarpa salpa*, *Trachurus trachurus* and *U. canariensis* (Table 4.1). Nineteen species (*Argyrosomus japonicus, Atherina breviceps, C. gilchristi, Caffrogobius nudiceps, Elops machnata, G. aestuaria, G. callidus, Lithognathus lithognathus, Chelon dumerili, Monodactylus falciformis, Pseudomyxus*

capensis, Omobranchus woodi, Ostracion cubicus, Platycephalus indicus, Pomadasys commersonnii, P. knysnaensis, Rhabdosargus thorpei, Syngnathus temminckii and *Torpedo fuscomaculata*) were only caught in the estuary and never in the marine environment (Table 4.1).

Table 4.1: Species composition, mean CPUE (fish per 1000 m^2), total number of individuals and the percentage contribution (%) of each species to the total catch of demersal fishes caught in the Algoa Bay marine nearshore, Sundays and Swartkops estuaries during the sampling period (July 2017 – September 2019). Habitat association: BP = bentho-pelagic, B = benthic, P = pelagic. Estuarine association category: M = Marine species, MS = Marine stragglers, MEO = Marine estuarine-opportunist, MED = Marine estuarine-dependents, E&M = Estuarine and marine, E&F = Estuarine and freshwater species, using the international classification scheme of Potter et al. (2015). Species caught in the marine nearshore and estuarine environment are shaded in green and are highlighted in bold text. Grey shading shows species only caught in one environment (nearshore marine or estuarine).

				Algoa Bay		Sundays		Swartkops	
Family	**Species**	**Estuarine category**	**Habitat association**	**Mean CPUE**	**n (%)**	**Mean CPUE**	**n (%)**	**Mean CPUE**	**n(%)**
Ariidae	***Galeichthys feliceps***	MEO	BP	9.0	308 (39)	3.3	65 (2)	-	-
Atherinidae	*Atherina breviceps*	E&M	P	-	-	6.1	120 (3)	0.1	1 (0.04)
Blenniidae	*Omobranchus woodi*	E&M	BP	-	-	-	-	0.3)	4 (0.1)
	Parablennius cornutus	M	BP	0.03	1 (0.2)	-	-	-	-
Carangidae	*Trachurus trachurus*	MS	P	0.1	4 (1)	-	-	-	-
Clupeidae	*Gilchristella aestuaria*	SE	P	-	-	27.6	546 (15)	1.6	24 (1)
	Sardinops sagax	MS	P	0.5	17 (2)	-	-	-	-
Cynoglossidae	*Cynoglossus capensis*	M	B	0.3	5 (1)	-	-	-	-
	Cynoglossus zanzibarensis	M	B	1.2	40 (5)	-	-	-	-
Dasyatidae	*Dasyatis chrysonota*	MS	B	1.0	29 (4)	-	-	-	-
Elopidae	*Elops machnata*	MED	BP	-	-	0.6	11 (0.3)	-	-
Engraulidae	*Engraulis capensis*	MS	P	0.2	6 (1)	-	-	-	-
Gobiidae	*Caffrogobius agulhensis*	MS	B	0.03	0.1	-	-	-	-
	Caffrogobius gilchristi	E&M	B	-	-	27.3	541 (14)	27	419 (16)
	Caffrogobius nudiceps	E&M	B	-	-	1.1	21 (1)	-	-
	Caffrogobius saldanha	E&M	B	0.5	1 (2.3)	-	-	0.1	2 (0.1)
	Glossogobius callidus	E&F	B	-	-	18.5	367 (10)	24.7	380 (14)
	Psammogobius knysnaensis	E&M	B	-	-	21.3	421 (11)	24.2	373 (14)
Gymnuridae	*Gymnura natalensis*	M	B	0.1	4 (1)	-	-	-	-
Haemulidae	*Pomadasys commersonnii*	MED	BP	-	-	8.8	174 (5)	2.4	37 (1)
	Pomadasys olivaceus	MEO	BP	5.2	181 (23)	1.1	22 (0.6)	0.3	5 (0.3)
Monodactylidae	*Monodactylus falciformis*	MED	BP	-	-	0.1	1 (0.03)	0.1	1 (0.04)
Mugilidae	*Chelon dumerili*	MED	BP	-	-	0.1	2 (0.1)	-	-

	Pseudomyxus capensis	MED	BP	-	-	0.1	1 (0.03)	-	-
Myliobatidae	*Myliobatis aquila*	MEO	B	2 (0.1)	0.3	-	-	-	-
Ostraciidae	*Ostracion cubicus*	MS	B	-	-	-	-	0.1	1 (0.04)
Platycephalidae	*Platycephalus indicus*	MEO	B	-	-	0.5	9 (0.4)	0.5	7 (0.3)
Rajidae	*Raja clavata*	M	B	1 (0.03)	0.1	-	-	-	-
	Raja miraletus	M	B	2 (0.1)	0.3	-	-	-	-
Rhinobatidae	*Acroteriobatus annulatus*	MS	B	2 (0.1)	0.3	-	-	-	-
Sciaenidae	*Argyrosomus inodorus*	M	BP	46 (1.3)	6	-	-	-	-
	Argyrosomus japonicus	MED	BP	-	-	0.8	15 (0.4)	-	-
	Atractoscion aequidens	M	BP	14 (0.4)	2	-	-	-	-
	Umbrina canariensis	M	BP	23 (0.7)	3	-	-	-	-
Soleidae	***Heteromycteris capensis***	MED	B	4 (0.1)	0.5	4.0	78 (2)	24.7	381 (14)
	Solea turbynei	MED	B	11 (0.3)	1.4	33.3	659 (18)	17.2	265 (10)
Sparidae	*Cheimerius nufar*	MS	BP	1 (0.03)	0.1	-	-	-	-
	Diplodus capensis	MEO	BP	7 (0.2)	1	0.3	6 (0.2)	0.7	10 (0.4)
	Lithognathus lithognathus	MED	BP	-	-	0.2	4 (0.1)	0.7	10 (0.4)
	Rhabdosargus globiceps	MEO	BP	18 (0.5)	2	8.4	166 (4)	6.3	97 (4)
	Rhabdosargus holubi	MED	BP	8 (0.2)	1	25.0	493 (13)	42.3	656 (25)
	Rhabdosargus thorpei	MEO	BP	-	-	0.1	1 (0.03)	-	-
	Sarpa salpa	MEO	BP	1 (0.0)3	0.1	-	-	-	-
	Spondyliosoma emarginatum	MEO	BP	22 (0.6)	3	0.7	13 (0.3)	0.1	2 (0.1)
Syngnathidae	*Syngnathus temminckii*	E&M	BP	-	-	-	-	0.1	1 (0.04)
Tetraodontinae	*Geneion honckenii*	MEO	BP	5 (0.1)	1	-	-	-	-
Torpedinidae	*Torpedo fuscomaculata*	MEO	B	-	-	0.1	2 (0.1)	-	-
Triglidae	*Chelidonichthys kumu*	MS	BP	9 (0.3)	1.1	-	-	-	-
	Unidentified			6 (0.2)	1	0.2	3 (0.1)	0.4	6 (0.2)

4.3.2 Fish community structure and fish assemblage – environment relationships

Overall, demersal fish assemblages were significantly shaped by changes in salinity, silt content, turbidity and organic content (Figure 4.2a). Both the linkage tree and nMDS analyses indicated a statistically significant dissimilarity at 99.6% (SIMPROF; $P < 0.05$) between fish communities in the marine nearshore environment and the estuarine environment, based on salinity and habitat (estuarine environment – salinity < 34.7 or nearshore marine environment - salinity > 34.7) (Figure 4.2a). Marine nearshore fish assemblages were dominated by marine species (M) and marine stragglers (MS), which were never recorded in the estuarine environment (Figure 4.3a). Marine estuarine-opportunists (MEO) were more abundant in the marine nearshore than the estuarine environment (Figure 4.3a). Species caught in the marine nearshore were predominantly benthic (Figure 4.3b). Six species *Pomadasys olivaceus*, *Cynoglossus zanzibarensis*, *Caffrogobius saldanha*, *Spondyliosoma emarginatum*, *Umbrina canariensis* and *Galeichthys feliceps* were indicators of fish assemblages in the marine nearshore (Figure 4.4). Estuarine fish assemblages were dominated by estuarine and marine species (E&M) as well as marine estuarine-dependent species (MED) and were mainly benthic and pelagic species (Figure 4.3a & b). The estuarine environment was characterised by *Psammogobius knysnaensis*, *Solea turbynei*, *Rhabdosargus holubi*, *Caffrogobius gilchristi*, *Glossogobius callidus* and *Heteromycteris capensis* (Figure 4.4).

Although estuarine fish assemblages in both the Sundays and Swartkops were very similar (48% similarity), estuarine fish assemblages separated into four groupings based on salinity, silt, turbidity and organic content (Figure 4.2a & b). Fish assemblages in the lower reaches of the Swartkops Estuary (SW1, SW2 and SW3) separated from the other sites of both estuaries, based on low silt content and turbidity and were characterized by *P. knysnaensis*, *H. capensis, S. turbynei* and *R. globiceps* (Figure 4.4). Two significantly distinct clusters (D) were further separated by salinity (< 22.9 or > 24), with fish assemblages in the middle and upper reaches of the Sundays (S5 – S9) and upper reaches of the Swartkops (SW6 and SW7) associated with low salinity (< 22.9) (Figure 4.2a). This grouping was characterised by *S. turbynei, R. holubi, G. aestuaria*, *G. callidus* and *Pomadasys commersonnii* (Figure 4.4). The lower reaches of the Sundays Estuary (S2, S3 & S4) were characterized by *S. turbynei, C. gilchristi*, *G. asetuaria*, *R. holubi*, *P. knysnaensis*, *G. feliceps* and *P. commersonnii* (Figure 4.4). The Sundays Estuary Site 1 (S1) was grouped with the middle reaches of the Swartkops Estuary (SW4 and SW5) and this cluster was characterised by *S. turbynei, P. knysnaensis, R. holubi, C. gilchristi* and *H.*

*capensi*s (Figure 4.2a & 4.4). Although, there were no significant differences in fish assemblages within the marine nearshore sites, the fish assemblage in Site 1 (A1) was relatively distinct from other sites (Figure 4.2a & b).

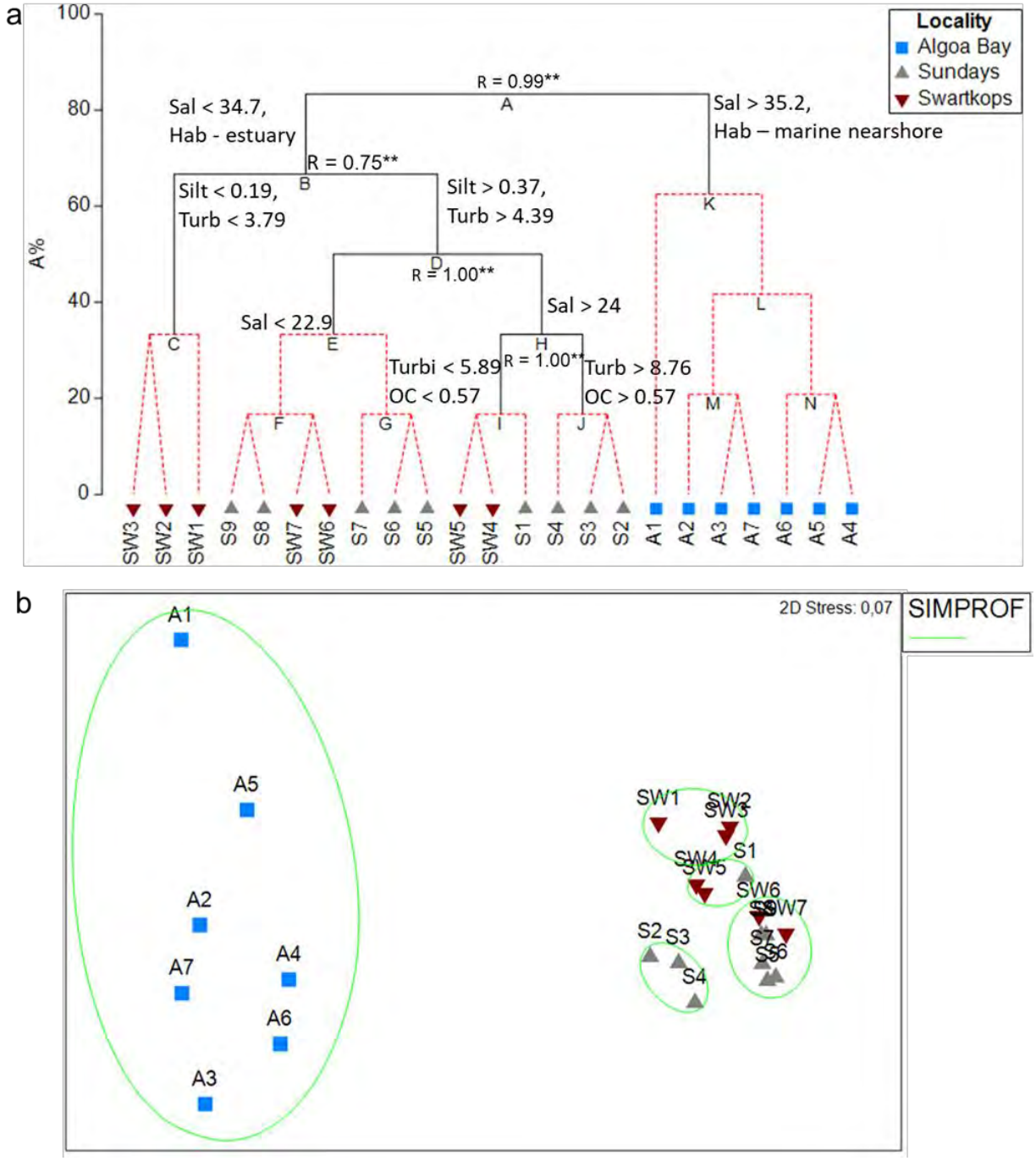

Figure 4.2: (a) Linkage tree dendrogram and (b) non-metric multidimensional scaling (nMDS) plot showing the fish community structure in Algoa Bay, Sundays and Swartkops based on the mean catch-per-unit-effort (CPUE) (fish per 1000 m^2) per site constrained by environmental variables during the study period (July 2017 – September 2019). The black lines in the dendogram indicates the significant clustering ($P < 0.01$). Green circles in the MDS plot delineate SIMPROF. The symbols and numbers represents site number (A = Algoa Bay, S = Sundays, SW = Swartkops). Abbreviation for the environmental variables (Sal = salinity, Turb = turbidity, OC = organic content) and Hab = habitat. For each significant branch, R value and environmental variables with values responsible for the clustering are given.

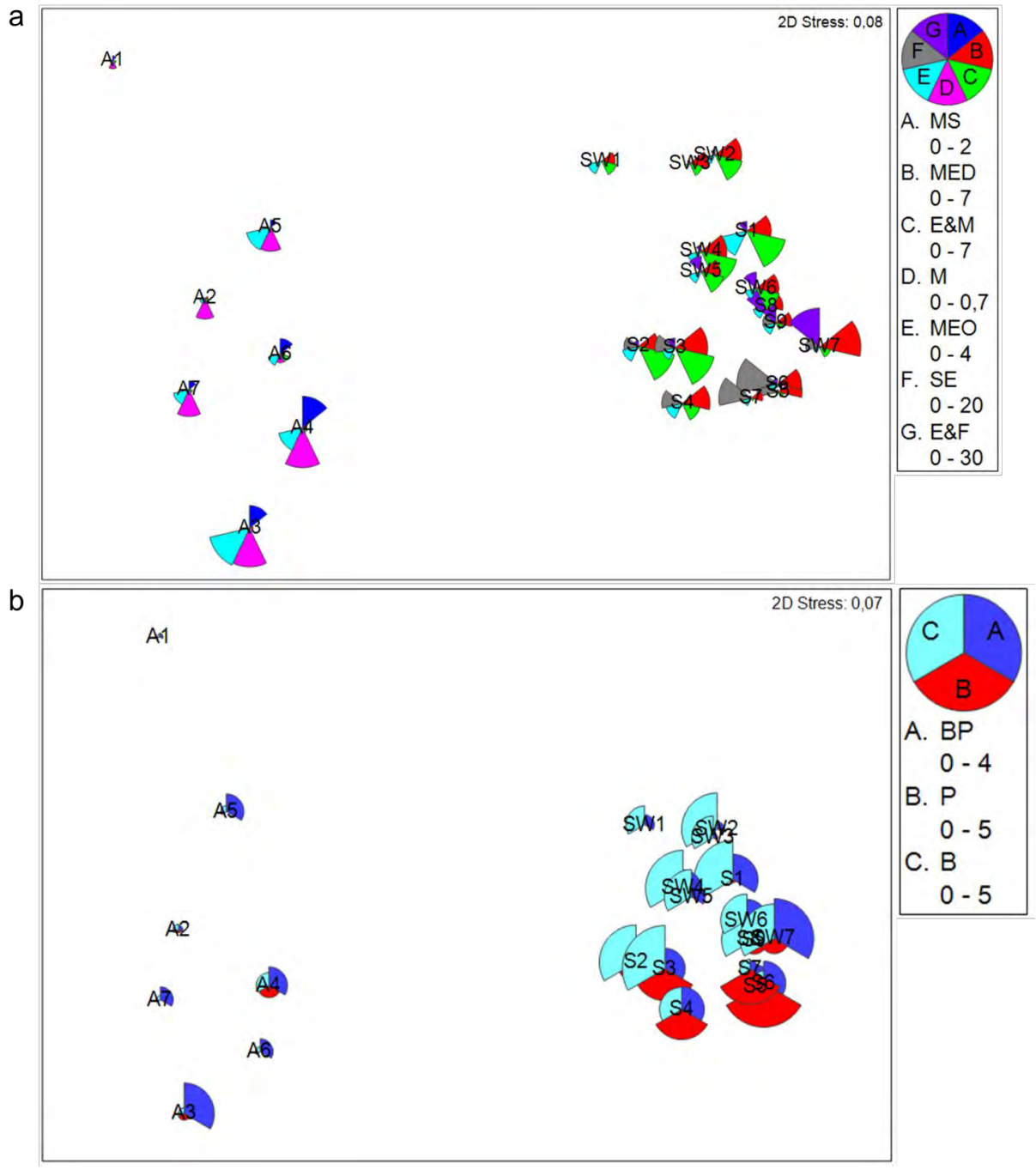

Figure 4.3: Pie charts created in the nMDS plot showing fish assemblages grouped into (a) fish guild (b) and habitat type in the Algoa Bay, Sundays and Swartkops sites during the sampling period (July 2017 – September 2019). The symbols represent estuarine association category (M- marine species, MS- marine stragglers, MEO- Marine estuarine-opportunist, MED- Marine estuarine-dependents, E&M- Estuarine and marine, SE- Solely estuarine, E&F- Estuarine and freshwater species and habitat association (BP = bentho-pelagic, B = benthic, P = pelagic).

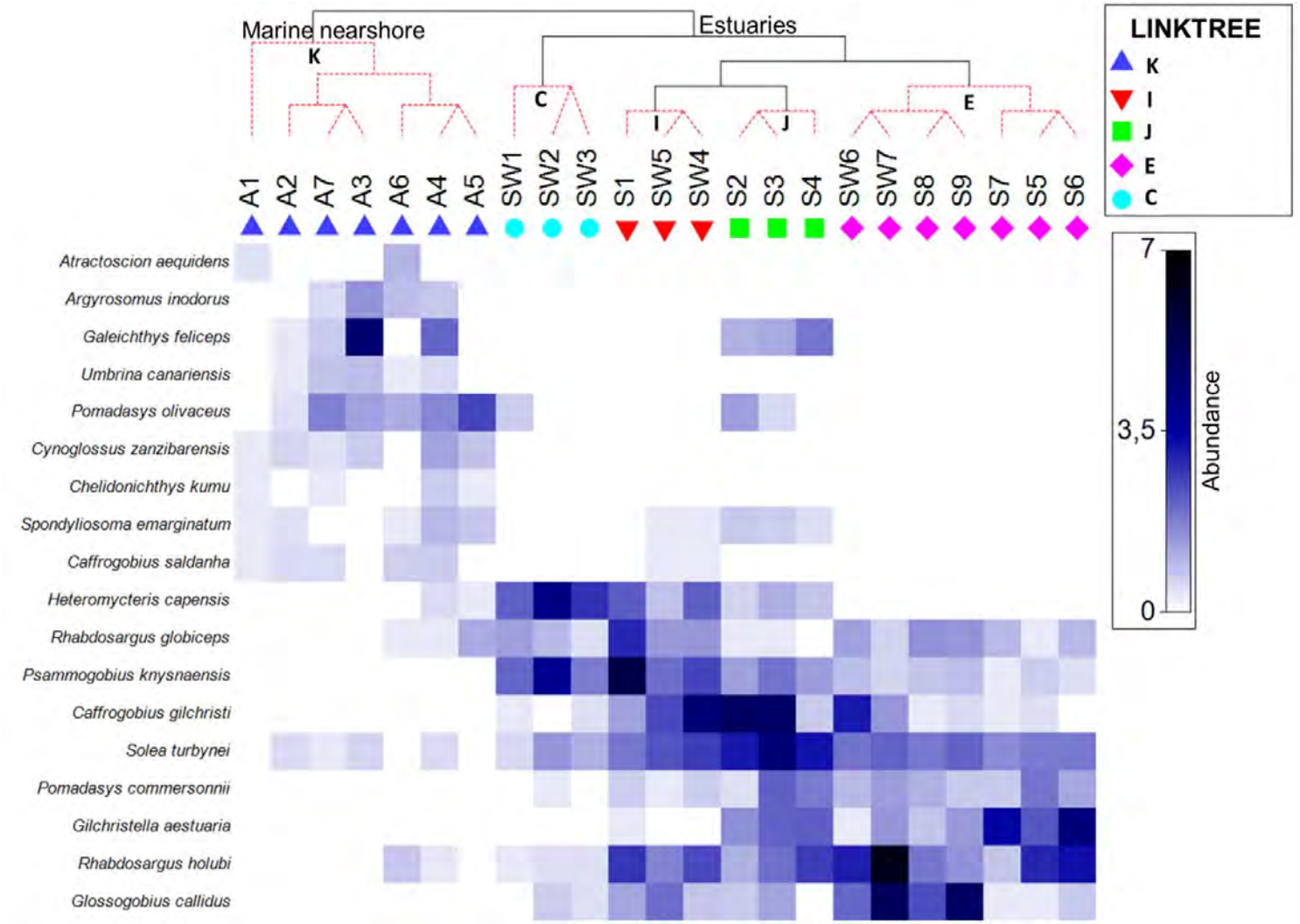

Figure 4.4: Shade plot showing the 18 most abundant fish species across the five LINKTREE statistically distinct SIMPROF groups recorded in the marine nearshore, Sundays and Swartkops estuaries during the sampling period (July 2017 – September 2019). Fish species have been clustered and ordered with species that have similar distribution across samples placed together based on the LINKTREE results. The abundance of each species (listed on the left) is represented by different colour scales, from white (absent) to navy blue (highest abundance).

4.3.3 Spatial variation in environmental variables and species assemblages across two different freshwater to marine seascapes

Sundays estuarine to marine seascape

In the Sundays estuarine to marine seascape, bottom salinity increased from the head of the estuary (Site S9) (2.5) to the marine nearshore sites (35.0). In the marine nearshore sites, bottom salinity ranged between 34.2 (Site A5) and 35.4 (Site A7) (Figure 4.5a). Water temperature in the marine nearshore sites ranged between 18.2 °C (Site A7) and 18.5 °C (Site A6). In the estuary, temperature showed an increasing trend from Site S1 (18.9 °C) in the mouth to Site S7 (20.7 °C) in the upper reaches (Figure 4.5b). Turbidity in the marine nearshore was highest at Site A7 (7.4 NTU). In the estuary, higher values (13.1 to 16.6 NTU) were recorded in the middle reaches (sites S5 and S6) compared to other sites (4.3 to 12.6 NTU) (Figure 4.5c). Dissolved oxygen in the marine nearshore was highest at Site A5 (8.0 mg/l), while in the estuary similar values were recorded at the mouth (8.0 mg/l) and very low values at Site S6 (4.7 mg/l) in the middle reaches (Figure 4.5d). The organic content in the sediment in the marine nearshore ranged from 0.4% at sites A6 & A7 to 0.5% at Site A5. In the estuary the mean organic content was high at sites S4 – S6 (ranging between 1.3 and 1.7%) (Figure 4.5e). In the marine nearshore, the highest silt content was recorded at Site A7 (0.8%). In the estuary the highest silt content was recorded in the lower reaches and ranged from 5.6% at Site S3 to 11% at Site S4 (Figure 4.5f).

Swartkops estuarine to marine seascape

In the Swartkops estuarine to marine transition, salinity increased from the head of the estuary (SW7) (21.6) to the marine nearshore sites (A1 – A4) (35.2) in the bottom. In the estuary, salinity was highest at Site SW1 (34.7) and lowest at Site SW7 (21.6) (Figure 4.5g). The mean water temperature was highest at Site SW7 (21.3 °C) in the upper reaches and lowest at Site SW1 at the mouth region (19.5 °C). In the marine nearshore sites, mean water temperature ranged from 17.2 °C (Site A1) to 18.7 °C (Site A4) (Figure 4.5h). Turbidity showed a decreasing trend from the upper reaches (SW6 – SW7) (9.5 – 10.3 NTU) of the estuary to the mouth region (SW1) (2.1 NTU). Higher mean turbidity values were recorded at Site A3 and A4 (7.4 – 7.6 NTU) and lower values at Site A1 and A2 (1.9 – 2 NTU) in the marine nearshore (Figure 4.5i). The highest dissolved oxygen in the marine nearshore was recorded at Site A4 (7.9 mg/l) and the lowest was observed at Site A3 (6.5 mg/l). In the estuary, dissolved oxygen concentrations increased from the head region (Site SW7) (4.2 mg/l) to the mouth region (SW1)

(7.6 mg/l) (Figure 4.5j). The mean organic content in the sediment in the marine nearshore ranged from a mean of 0.5% recorded at Site A3 to 3.6% at Site A1. In the estuary, the mean organic content ranged between 0.3% (sites SW1, SW3 and SW6) and 0.8% (Site SW4) (Figure 4.5k). The silt content of the sediment ranged from 0.05% (Site A2) to 13% (Site A3) in the marine nearshore. In the estuary, silt content ranged from 0% (sites SW1 and SW3) to 17% (Site SW7) (Figure 4.5l).

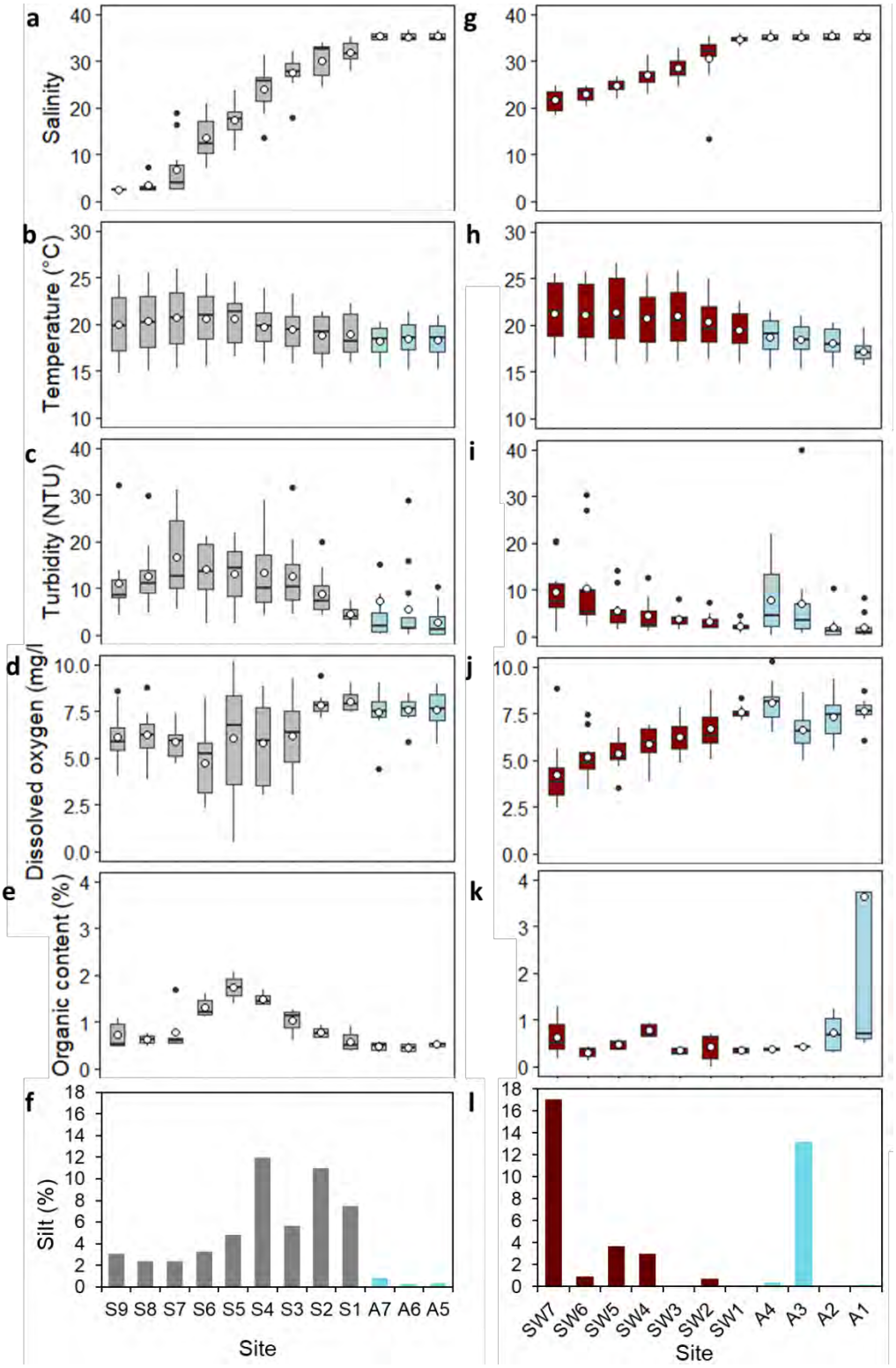

Figure 4.5: Boxplots showing environmental variables (salinity, turbidity (NTU), temperature (°C), dissolved oxygen (mg/l), organic content (%) and silt content (%)) recorded in the (a– f) Sundays freshwater to marine seascape and (g – l) Swartkops freshwater to marine seascape during the sampling period (July 2017 – September 2019). Boxes represent the upper and lower limits of the third and first quartiles; whiskers represent the range; thick solid lines the median. Black circles are outliers and white circles represent mean.

Overall mean catch-per-unit-effort (CPUE) (in each site throughout the sampling period) in the Sundays Estuary increased from the upper reaches (93 – 178 fish per 1000 m^2) to the lower reaches (229 – 355 fish per 1000 m^2) and then decreased substantially (13 – 18 fish per 1000 m^2) in the marine nearshore environment. The number of species varied between 8 and 10 in the upper reaches (S7 to S8), and 9 to 17 in the middle reaches (S3 to S6) and 14 to 16 in the lower reaches (S1 to S2) of the Sundays Estuary. In the marine nearshore, the number of species ranged from 12 (A6 and A7) to 14 (Site A5) (Figure 4.6a). Marine estuarine-dependent and SE species dominated the middle reaches of the estuary, with the lower reaches comprised mostly of E&M and MED species. The upper reaches were dominated by the E&F and E&M species. Marine nearshore sites (A5, A6 & A7) were dominated by MEO species (Figure 4.6b). In terms of habitat association, benthic species dominated the lower reaches and the head (Site S9) of the estuary, with the highest catches of bentho-pelagic and pelagic species recorded in the middle reaches. In the marine nearshore, bentho-pelagic species dominated in all the sites (Figure 4.6c).

In the Swartkops freshwater to marine transition, mean CPUE (in each site throughout the sampling period) decreased from the upper reaches (387.7 fish per 1000 m^2) to the lower reaches (62.7 fish per 1000 m^2) and increased in the marine nearshore Site A3 and A4 (30.0 – 47.0 fish per 1000 m^2) (closer to the estuary mouth) before decreasing again in Sites A1 and A2 (2.5 – 2.6 fish per 1000 m^2) (far from the estuary mouth) (Figure 4.6d). The number of species (over the whole samoling period in each site) varied between 6 (Site A1) and 16 (Site A4) in the marine nearshore and ranged from 9 in the lower reaches (Site SW2) to 14 in the middle reaches (Site SW4) of the estuary (Figure 4.6d). These were dominated by MED and E&M species throughout the estuary. In the marine nearshore, MEO species dominated sites A1, A3 and A4 with M species dominating in Site A2 (Figure 4.6e). Benthic and bentho-pelagic species dominated the mouth region (SW1) and the head region (SW7) of the estuary, while other sites comprised mainly benthic species. The nearshore species were mostly bentho-pelagic, with pelagic species also dominating at Site A4 (Figure 4.6f).

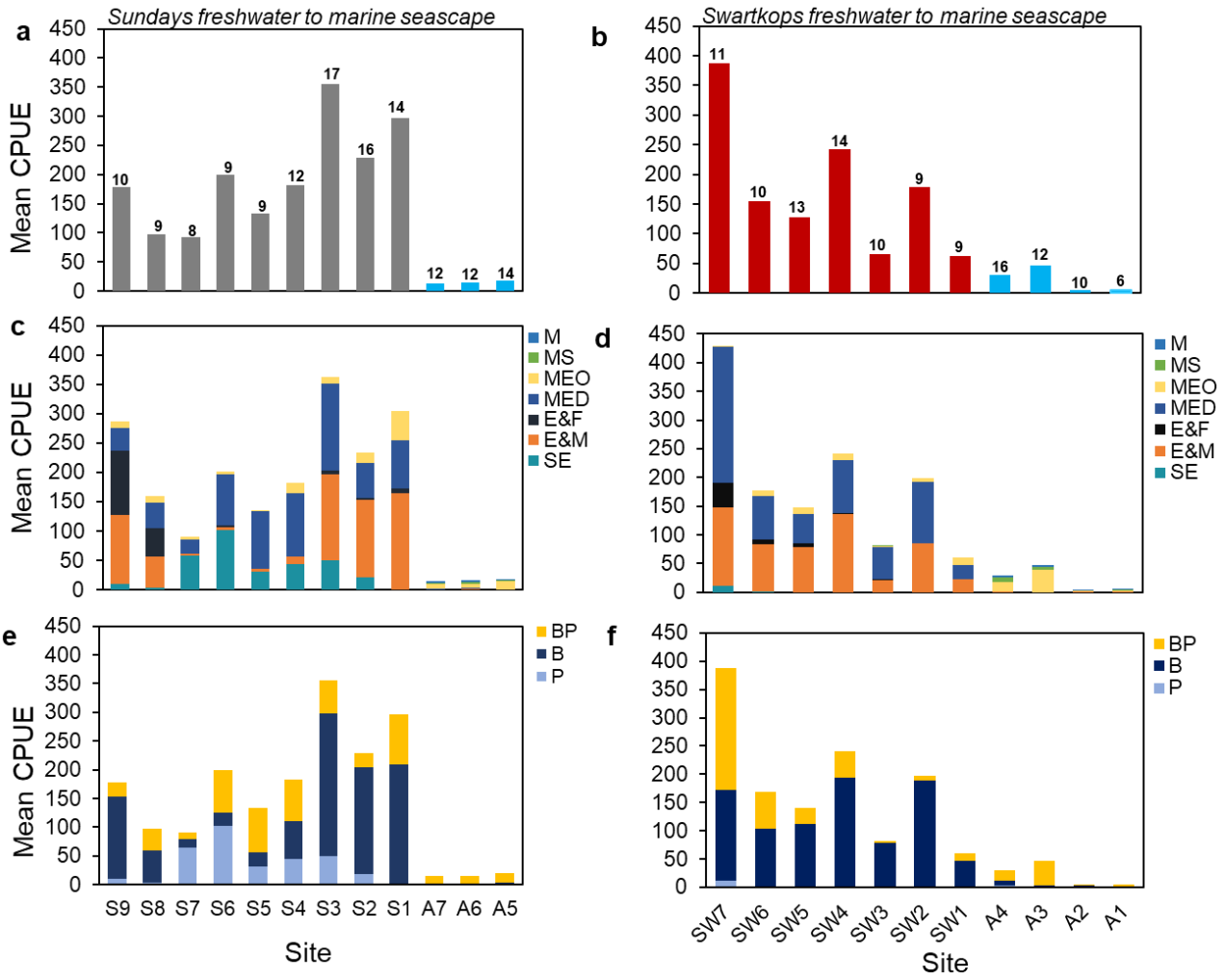

Figure 4.6: The mean catch-per-unit-effort (CPUE) (fish per 1000 m^2) recorded at each site in the Sundays freshwater to marine seascape (a, c & e) and Swartkops freshwater to marine seascape (b, d & f) during the sampling period (July 2017 – September 2019). Habitat association: BP = bentho-pelagic, B = benthic, P = pelagic. Estuarine association category: M = marine species, MS = marine stragglers, MEO = Marine estuarine-opportunist, MED = Marine estuarine-dependents, E&M = Estuarine and marine, E&F = Estuarine and freshwater species and SE- Solely estuarine. Number above each bar indicates the total number of species caught in each site.

4.3.4 Seasonal variation in fish abundance (CPUE)

In all sites (from the upper reaches of both estuaries to the marine nearshore), the highest catches were recorded in summer and spring and the lowest catches in winter (Figure 4.7a&b). High catches were also observed in autumn, particularly in the head region of the Swartkops (Mean CPUE: SW7 = 472 fish per 1000 m^2). In the Sundays Estuary, autumn catches decreased

from the lower (Site S3) (Mean CPUE = 301.6 fish per 1000 m^2) to the upper reaches (Site S7) (Mean CPUE = 87.5 fish per 1000 m^2) (Figure 4.7a & b).

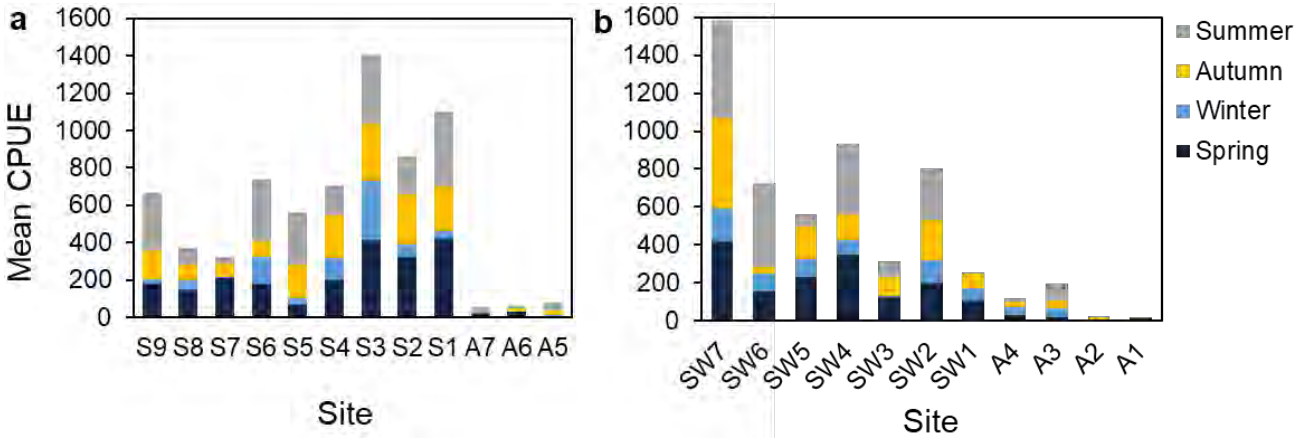

Figure 4.7: The mean catch-per-unit-effort (CPUE) (fish per 1000 m^2) recorded seasonally in the (a) Sundays Estuary with the adjacent marine nearshores sites (Sites A5, A6 & A7) and (b) Swartkops Estuary with adjacent marine nearshore sites (Site A1, A2, A3 & A4) during the sampling period (July 2017 – September 2019).

4.3.5 Correlation between abundant species and environmental variables

In the marine nearshore, for the most abundant species, the only significant spearman rank correlation observed between CPUE and environmental variables was between *A. inodorus* and silt content (Table 4.2). *Solea turbynei*, *R. holubi, G. aestuaria*, *G. callidus* and *P. commersonnii,* which dominated the upper reaches of both estuaries, showed a significant negative correlation with salinity. *Rhabdosargus holubi, P. commersonnii, S. turbynei* and *G. asetuaria*, which characterised the lower reaches of the Sundays Estuary (S2, S3 and S4), showed a significant positive correlation with turbidity and organic content. *Psammogobius knysnaensis* and *H. capensis* dominated the lower reaches of the Swartkops Estuary and had a significant negative correlation with turbidity and silt. *Psammogobius knysnaensis* and *C. gilchristi* had significant negative correlations with turbidity and organic content in both estuaries and these species dominated the lower reaches (Site S1) of the Sundays Estuary and the middle reaches (SW4 and SW5) of the Swartkops Estuary (Table 4.2).

Table 4.2: Spearman rank correlation coefficient of the most abundant species caught in the marine nearshore, Sundays and Swartkops estuaries and the environmental variables. (*$P <$ 0.05, **$P < 0.001$). DO = Dissolved oxygen, Temp = Temperature. Habitat association: BP = bentho-pelagic, B = benthic, P = pelagic. Estuarine association category: M = Marine species, MS = Marine stragglers, MEO = Marine estuarine-opportunist, MED = Marine estuarine-dependents, E&M = Estuarine and marine, E&F = Estuarine and freshwater species.

	Fish guild	Habitat association	n	Salinity	Turbidity	Temp	DO	Organic content	Silt	Wave height
Algoa Bay										
A. inodorus	M	BP	37	-0.04	-0.07	0.08	-0.19	-0.09	0.38**	-0.04
C. zanzibarensis	M	B	40	-0.09	-0.02	-0.17	0.12	0.00	-0.08	-0.19
G. feliceps	MEO	B	308	-0.05	-0.03	-0.09	0.04	-0.08	0.21	-0.09
P. olivaceus	MEO	BP	162	0.10	-0.11	0.02	-0.12	-0.07	0.12	0.18
Sundays										
C. gilchristi	E&M	B	541	0.32**	-0.10	-0.09	0.23*	-0.06	0.29**	
G. callidus	E&F	B	367	-0.36**	-0.02	0.18	-0.09	-0.25*	-0.25*	
P. knysnaensis	E&M	B	421	0.34**	-0.25*	-0.07	0.27**	-0.21*	0.15	
R. holubi	MED	BP	489	0.05	0.03	-0.03	0.06	0.25*	0.01	
S. turbynei	MED	B	659	0.23*	0.16	-0.05	0.01	0.05	0.22*	
G. aestuaria	SE	P	546	-0.05	0.11	0.20	-0.10	0.01	-0.74	
P. commersonnii	MED	BP	174	0.13	0.13	0.19	-0.23*	0.29**	0.06	
Swartkops										
C. gilchristi	E&M	B	419	-0.13	0.05	0.29*	0.06	0.09	-0.04	
G. callidus	E&F	B	380	-0.38**	0.09	0.09	-0.32**	0.23	0.62**	
P. knysnaensis	E&M	B	373	0.31**	-0.10	-0.03	0.19	0.01	-0.22*	
R. holubi	MED	BP	656	0.32**	0.39**	0.22	-0.40**	0.24*	0.12	
S. turbynei	MED	B	265	-0.19	0.23*	-0.03	-0.23*	0.18	0.12	
H. capensis	MED	B	381	0.36**	-0.15	0.06	0.25*	-0.12	-0.24*	

4.4. Discussion

In the present study, habitat type (estuarine versus marine nearshore) characterised by salinity had the greatest influence on the composition of demersal fish assemblages in the Algoa Bay study area, with two discrete demersal fish assemblages identified representing the soft-bottom estuarine and marine nearshore environments. The marine nearshore fish assemblage was mainly comprised of bentho-pelagic and benthic species, with marine estuarine-opportunists (MEO) (*P. olivaceus*, *G. feliceps*) and marine species (MS & M) (*A. inodorus* and *C. zanzibarensis*) dominating catches. The estuaries were dominated by benthic marine estuarine-dependents (MED) and marine and estuarine species (E&M) (*R. holubi*, *S. turbynei*, *H. capensis*, *P. knysnaensis* and *G. callidus*).

Different demersal fish species dominate in each environment in the estuarine and marine nearshore environments (e.g. Prista et al 2003; Guerreiro et al. 2021). The composition of demersal species in the marine nearshore of Algoa Bay was similar to earlier studies conducted in the same region and at similar depth (Wallace et al. 1984a, c; Beckley 1984; Beckley 1986). For example, *G. feliceps, P. olivaceus* and *Argyrosomus sp*. also dominated the marine nearshore fish composition of Algoa Bay in earlier studies (Lasiak 1983; Buxton et al. 1984; Wallace et al. 1984a). Beckley (1984) sampled the area off the Swartkops Estuary mouth with a trawl net and in this study, *P. olivaceus* was the dominant species caught, followed by *Argyrosomus sp*. Watling and Watling (1983) attributed the higher abundance of these species in the marine nearshore region of Algoa Bay compared to other areas along the Cape south coast (St Francis Bay, Plettenberg Bay, Mossel Bay and St Sebastian Bay) to the fact that, Algoa Bay forms part of a transitional region between the warmer subtropical and cooler temperate zone, which makes it a very productive and important ecological region. The estuarine (both the Sundays and Swartkops estuaries) demersal fish composition was similar to those recorded in other south-eastern coast estuaries, where *S. turbynei*, *H. capensis*, *R holubi*, *G. callidus* and *P. knysnaensis* also dominate the demersal fish assemblage (e.g. Richardson et al. 2006; Bailey and James 2013; Nodo et al. 2017, 2018).

Species richness and the extent of overlap among species varies between marine and estuarine environments and between biogeographic regions (e.g. Potter et al. 1990) owing to changes in environemnetal factors and the availability of suitable habitats in both environments. In the present study, of the 48 species recorded during the study period, 20 were only caught in the

nearshore marine environment and 19 in the estuarine environment and nine species were caught in both. Contrary to findings of the current study, in temperate south-western Australia, several studies have found that the number of species restricted to the estuarine environment is higher than the marine nearshore, with overlap between the two habitats being greater. For example, in the Laschenault Estuary and Koombana Bay, out of 50 species caught, 16 species were restricted to the estuary and eight were only recorded in the bay with 26 species common to both habitats (Potter et al. 1997). Similarly, in the Blackwood River Estuary and adjacent Flinders Bay, a total of 49 species were caught with 23 species recorded only in the estuary, seven were recorded only in the bay and 19 species were found in both habitats (Valesini et al. 1997). The differences between this study and those conducted in temperate south-western Australia could be attributed to the presence of suitable similar, sheltered alternative nursery habitats in the marine nearshore environments in south-western Australia than in southern Africa (Potter et al. 1997).

The demersal fish community structure from the head region of the estuary to the marine environment is determined by a number of physical drivers including salinity, sediment characteristics and turbidity (e.g. Martino and Able 2003). In the present study, salinity was found to be the primary driver of fish community groupings in the two freshwater to marine demersal seascapes. Fish communities in the upper reaches of both estuaries (freshwater/estuarine ecotone) differed from those in the rest of the estuary, based on low salinities, while the fish communities in the lower reaches of the Sundays Estuary were associated with high euhaline salinities. Salinity gradients have been shown to influence the structure of fish assemblages in coastal and estuarine waters, with particular species dominant in waters with different salinity ranges (e.g. Potter and Hyndes 1999; Martino and Able 2003; Harrison and Whitfield 2006). The role of salinity is hugely important in estuarine waters (the most important of all drivers) (Harrison and Whitfield 2006) however, this effect is not as prominent in coastal waters where salinity range is narrow (Miró et al. 2020).

Sediment (high sand content) and low turbidity were also important drivers of fish communities in the lower reaches of the Swartkops Estuary. The lower reaches of the Swartkops Estuary were dominated by *P. knysnaensis* and *H. capensis*. The distribution and abundance of these two species in estuaries is linked to sediment characteristics, where they are both known to prefer the sandy sediment, characteristic of the mouth region of most South African estuaries (e.g. Ter Morshuizen and Whitfield 1994; Richardson et al. 2006; Nodo et al. 2018). Sediment

type has been described as a major factor related to the distribution and abundance of demersal fish species in the marine nearshore (Prista et al. 2003) and the estuarine environments (e.g. Stoner et al. 2002; Richardson et al. 2006). Gibson (1994) suggested that sediment type is a critical factor determining the fine scale distribution of demersal/benthic fishes because they live in direct association with the sediment and because sediment provides both food and shelter from predators. One of the main reasons why sediment type is important for the distribution of many benthic or bentho-pelagic fish species is because many of these species feed on benthic invertebrates and the distribution of the latter is highly dependent on the sediment type (Gibson 1994; Nicolas et al. 2007).

The abundance of *R. holubi, S. turbynei* and *P. commersonnii* was positively associated with low salinity and high turbidity in the Swartkops and Sundays estuaries. These species are known to prefer turbid (> 10 NTU) and low saline waters (salinity < 20) in estuaries (Whitfield et al. 1994). *Glossogobius callidus* distribution and abundance in the Swartkops and Sundays estuaries was also linked to low salinity, high turbidity and high silt. Similar distribution patterns of this species have been recorded in other South African estuaries, with this species preferring the turbid, low salinity upper reaches (Beckley 1983; Ter Morshuizen and Whitfield et al. 1994; Richardson et al. 2006).

In the marine nearshore environment, and among the most abundant species, only one species showed a significant relationship with the recorded/selected environmental variables. The important fishery species, *A. inodorus* was positively associated with high silt content of the sediment. In South Africa, this species is known to prefer nearshore environments with sandy and muddy bottoms (Griffiths 1996; 1997), as well as soft-bottom bay areas in depths less than 50 m (Heemstra and Heemstra 2004). In earlier studies *A. inodorus* was among the most abundant species at depths ranging from 5 to 7 m deep in Algoa Bay (Beckley 1984; 1990) in the soft substratum regions. The fact that no significant relationships between the environmental variables and the other abundant species recorded in the marine nearshore, which include *C. zanzibarensis, G. feliceps* and *P. olivaceus,* suggest that there may be other variables not recorded in this study which influence the distribution and abundance of these species in the marine nearshore environment. Some of these variables may include abiotic factors such as food or prey availability and predation. It could also be due to the fact that the range of the recorded environmental variables is narrow and not wide enough for differences in CPUE of these species to be noticeable.

Fish abundance differed across different seascapes with fish assemblages either decreasing from the head region of the estuary to the marine nearshore environment or increasing in the same direction. Indeed, the Sundays and Swartkops freshwater to marine seascape had different fish abundance trends. In the Sundays freshwater to marine seascape, abundance increased from the upper reaches to the mouth region and then decreased substantially in the adjacent marine nearshore environment. In contrast, in the Swartkops freshwater to marine seascape, abundance decreased from the head to the mouth and then increased again in the marine nearshore environment, in sites closer to the estuary mouth and decreased again further from the estuary mouth. These differences could be related to the hydro-geomorphological, physicochemical and biological characteristics of the two different systems. According to Whitfield (1999) no two estuaries are the same in terms of their biological and physical characteristics as the quantity and quality of their habitats are often diverse. Indeed, the Swartkops Estuary has extensive mudflats, shallow creeks and saltmarshes that provide refuge for fishes, while the Sundays Estuary is channel-like, with steep banks and muddy sediment, with little or no seagrass (Baird et al. 1988; Scharler and Baird 2005). Riverine influences and anthropogenic effects (as both estuaries are highly polluted) have also been found to contribute to differences in estuarine fish distribution and habitats along adjacent coastal regions (Bennett 1989). The differences observed in the demersal soft-bottom fish assemblage along the freshwater to marine continuum in both systems were primarily related to salinity gradients. In the Sundays freshwater to marine continuum, a well-developed horizontal salinity gradient was observed followed by higher fish abundance and diversity (species richness) in the lower reaches of the estuary. In the Swartkops, however, salinity gradients were not as pronounced (polyhaline upper reaches), with higher fish abundance in the upper reaches and higher diversity in the middle reaches. Furthermore, salinities recorded in the upper reaches of the Swartkops were similar to the middle reaches of the Sundays, suggesting that differences between the two systems are driven by salinity gradients. According to Whitfield (2019), in estuaries with ‘normal’ salinity gradients, fish composition and abundance along the gradient tend to change in response to salinity. In addition, it has been suggested that, larger estuaries with high freshwater input have higher primary productivity and greater food availability, which may result in nutrient rich adjacent marine nearshore waters (Prista et al. 2003). Therefore, marine nearshore structure and functioning also differ depending on the type of the adjacent estuarine environment.

Miró et al. (2020) in the Gulf of Cadiz (south-west Iberian Peninsula), compared four estuaries with their adjacent marine nearshore and related the differences observed among estuaries to the salinity gradients in each system. They found that higher fish abundance and diversity (species richness) were observed in low saline areas in estuaries that had long transition zones and well-defined salinity gradients (Guadalquivir and Guadiana estuaries) compared to other estuaries (Odiel-Tinto and Cadiz estuaries). Martino and Able (2003) also related differences in species richness within the ocean–estuarine ecotone to salinity gradients.

The abrupt decrease in CPUE in the soft-bottom marine nearshore environment compared to the soft-bottom estuarine environments may be related to environmental characteristics and the productive nature of estuarine ecosystems (Martino and Able 2003; Miró et al. 2020). It could also be noted that although the area sampled between the two environments was comparable, the extent of the area sampled/trawled in the marine nearshore compared to available habitat in the marine environment was far less than the estuary (where the entire estuary was sampled). Although the Swartkops estuarine/marine ecotone did not really extend into the marine nearshore, the proximity of the estuary did affect the abundance of fish adjacent to the Swartkops Estuary as the CPUE in the nearshore sites adjacent to the Swartkops was higher closer to the estuary mouth than the far sites. While in the Sundays nearshore, CPUE did not show much variation among the sites. This suggests that the Swartkops estuarine environment has some influence on fish assemblages and environmental characteristics in the immediate vicinity of the estuary.

While this chapter has provided much needed information on the overall demersal fish assemblage and environmental factors driving their distribution in the estuarine and marine nearshore environment of Algoa Bay, understanding the role of these different seascapes in the life history of the demersal fish assemblage is critical in the identification of nursery and/or essential fish habitat. As a result, the next chapter focuses on different life stages and nursery role of the estuarine and nearshore marine environment.

CHAPTER FIVE

The nursery function of shallow nearshore and estuarine benthic habitats of Algoa Bay for demersal fishes

5.1 Introduction

Estuaries play a critical role in the early life history of marine fish including many economically and ecologically important species (e.g. Lenanton 1982; Potter et al. 1990; Beck et al. 2001; Able 2005; Litvin et al. 2018). Research throughout the world has shown that estuarine fish communities are dominated by the juveniles of marine species (Potter et al. 1990; Whitfield 1999; Martinho et al. 2007), which make them important nursery grounds (e.g. Beck et al. 2001; Able 2005; Dahlgren et al. 2006; Nagelkerken et al. 2015; Sheaves et al. 2015). The importance of estuaries for the early-life stages of fishes has been linked to food provision and protection from predation and favourable environmental conditions for growth, such as high temperatures (e.g. Boehlert and Mundy 1987; Potter and Hyndes 1999; Elliott 2004; Gillanders 2005). Marine nearshore environments also serve as nursery areas for a wide variety of marine fishes, including species common to adjacent estuarine and/or offshore (deeper areas/open ocean) areas, but also species characteristic of the marine nearshore, not present in nearby marine or estuarine habitats (Lenanton 1982; Ross et al. 1987; Clark et al. 1994; Blaber et al. 1995). Less research attention has, however, focused on the nursery function of the marine nearshore environment (reviewed in Woodlands et al. 2012; Whitfield and Pattrick 2015) compared to estuaries. To fully understand the functional significance of both environments alone and relative to each other as settlement and nursery areas, it is important to concurrently study different life stages of fish from the head of the estuary to the adjacent marine nearshore environment (e.g. Valesini et al. 1997; Able et al. 2011).

This approach not only provides a quantitative understanding of the degree of estuarine use or estuarine dependence of species (e.g. Blaber et al. 1995; Martinho and Able 2003; Able et al. 2006; Woodlands et al. 2012), but also allows for the identification of critical transient settlement habitats, which may form population bottlenecks for species (e.g. Nagelkerken et al. 2015). Relatively few studies have, however, adopted this approach to assess the comparative nursery function of estuarine and marine environments (e.g. Valesini et al. 1997; Martino and Able 2003; Able 2005; Able et al. 2006; Woodland et al. 2012). In the northern

Hemisphere, Woodland et al. (2012) compared the nursery role of the benthic marine nearshore (5 – 10 m deep) and estuarine environment in Chesapeake Bay (temperate USA) for demersal fishes. The results revealed that both environments had a similar species composition and were dominated by juveniles indicating the nursery role of both environments. Similarly, in Great Bay (temperate USA), when looking at settlement and juvenile stages across an estuary-ocean ecotone, it was found that many species occurred as settlement stages and juveniles in both estuarine and marine nearshore benthic environments (Able 2005; Able et al. 2006).

In South Africa, although considerable research has focussed on the estuarine association and estuarine dependence of marine species (e.g. Wallace et al. 1984a; Beckley 1985; Whitfield 1994), much of this research has focussed on littoral areas in estuaries and intertidal and subtidal areas in the marine environment (reviewed in Whitfield 2019). Furthermore, no studies have used an estuary-ocean approach to examine settlement and nursery use of the early-life stages (late-stage larvae to juveniles) across the estuary-ocean transition in soft-bottom habitats. Results from Chapter Four (in this thesis) show that the demersal fish assemblage in the channel of the Sundays and Swartkops estuaries differed significantly from that of the marine nearshore zone, with relatively few species found in both environments. In order to interrogate the settlement and nursery function of each environment, it is essential to further examine fish assemblages in both environments by life stage.

The main aim of this chapter was therefore to determine the relative roles of the soft-bottom, benthic, marine nearshore and estuarine environments as settlement and nursery areas for demersal fishes. To achieve this, the specific objectives of this chapter were to determine (i) fish abundance (by life stage) in these two environments (ii) species composition (by life stage) in these two environments and (iii) patterns of habitat use (by life stage) across the estuary-ocean gradient in Algoa Bay.

5.2 Materials and Methods

5.2.1 Sampling

Demersal fishes were sampled from the marine nearshore environment (5 – 10 m depth) in Algoa Bay as well as from the channel of the Sundays and Swartkops estuaries; for twelve sampling trips between July 2017 and September 2019 (July 2017, February 2018, March 2018, July 2018, May 2018, August 2018, October 2018, November 2018, January 2019, February 2019, April 2019 and September 2019). Study site and sampling methods are described in detail in Chapter Two and Three.

5.2.2 Data analysis

Individuals were categorized into five life stages: postflexion larvae, transformation, Young-of-the-Year (YOY) juveniles, late juveniles and adults (mature stages) using the classification scheme outlined in Chapter Three.

All fish species caught were categorised into estuary-association guilds (Potter et al. 2015; Whitfield 2019) and categories using the updated Whitfield (1994, 2019) classification scheme detailed in Table 5.1. The classification scheme by Whitfield (1994, 2019) was used in this chapter to determine whether the classification of the species caught remains the same or needed to be changed based on the findings.

Table 5.1: Estuary-association categories of southern African fish fauna (Whitfield 1994, 2019).

Category	Description of categories
I	Estuarine species which breed in southern African estuaries. This category includes resident species that spawn only in estuaries, as well as species that also have marine or freshwater breeding populations:
IIa	Marine species with juveniles highly dependent on estuaries as nursery areas
IIb	Juveniles occur mainly in estuaries but are also common at sea
IIc	Juveniles sometimes occur in estuaries but are more abundant at sea
III	Marine straggler: species which occur in estuaries in very small numbers and are not dependent on these systems.
IV	Freshwater migrant species includes a few species which may breed in both freshwater and estuarine systems.
V	Catadromous species use estuaries as transit routes
M	Marine species do not occur in estuaries and occur only in the marine environment.

As in Chapter Four, catch-per-unit-effort (CPUE) was calculated as the index of abundance (fish per 1000 m^2) as per Harrison et al. (2017):

$$CPUE = \ total\ number\ of\ individuals\ at\ \ each\ site \div area\ trawled\ \times 1000$$

Given the similarities in fish assemblages between the two estuaries (Chapter Four), data from each estuary were pooled to compare the marine nearshore and estuarine habitats. To determine the catch contribution by life-stage (of all the species) in each habitat the total CPUE for each life-stage recorded throughout the study period was divided by the total CPUE by habitat, and graphically represented as the percentage abundance using a stacked bar graph.

To assess differences in CPUE among lifestages and habitat, and similarly among seasons and habitat, a two-way ANOVA was used. Diagnostics plots on the model residuals were run to assess the ANOVA assumptions, followed by the Shapiro-Wilk test of normality and Levene's test of homogeneity of variance. Since the assumptions of normality and homogeneity of variance were violated, the dependent variable was $\log_{10}(x+1)$ transformed. Post-hoc pairwise multiple comparisons were performed using Tukey's HSD test when there were significant differences ($P < 0.05$) among seasons and habitats to determine which means were significantly different from one another. These analyses were conducted using the '*mass*' package in the R environment for computing statistics version 3.6.0 (R Development Core Team 2019).

To determine the species composition by life-stage in each habitat, the number of species caught in the estuarine, nearshore marine and both habitats by life stage (settlement, juveniles and adults) was graphically represented as percentage taxa using a stacked bar graph. For this comparison, species were grouped into three ontogenetic stages, which include settlement (post flexion and transformation), juveniles (made up of YOY and late juveniles) and adults.

To determine spatial patterns in mean CPUE of the different life stages of the most abundant species (grouped into fish guilds) caught in the estuarine and marine nearshore environment; species were broadly grouped into the ontogenetic stages of settlement (post flexion and transformation), juveniles (made up of YOY and late juveniles) and adults. ArcMap (ArcGIS v. 10.3.1) was used to spatially visualize the abundance (measured as CPUE). Spatial analyses were carried out using conversion tools (points to raster files) available in the Arc Toolbox of ArcGIS, whereby point data were converted into a raster format.

5.3. Results

5.3.1. Fish abundance (CPUE) by life stage in the marine nearshore and estuary

In the marine nearshore, YOY juveniles dominated the overall catch (in terms of abundance), comprising 63% of the catch, followed by transformation (13%), late juvenile (11%) and then postflexion stages (10%). Very few adults were caught in the marine nearshore environment. In the estuarine environment, YOY juveniles (40%) also dominated the catch followed by adults (32%), postflexion (15%), transformation (9%) and late juvenile (5%) stages (Figure 5.1).

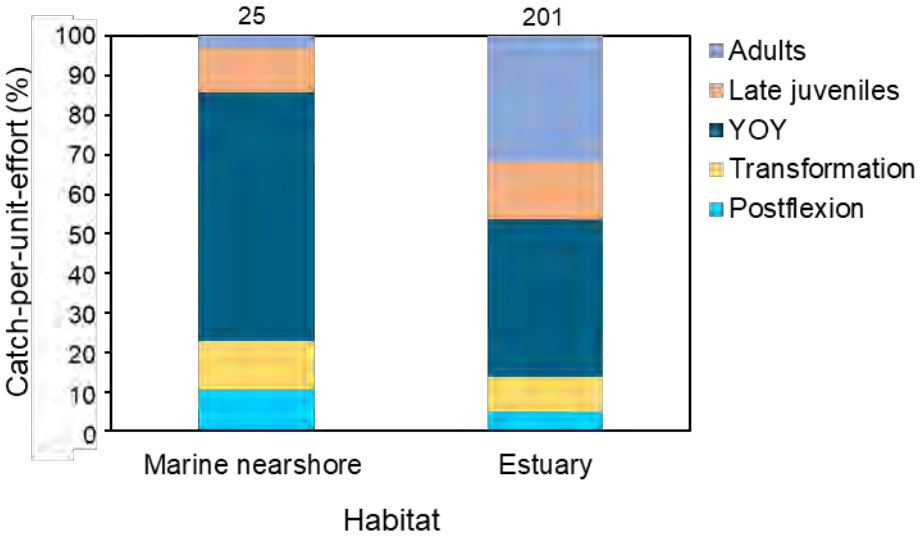

Figure 5.1: Percentage contribution of different life stages (%) (postflexion, transformation, Young-of-the-Year, late juveniles and adults) to the total abundance (catch-per-unit-effort (CPUE) (fish per 1000 m^2)) caught in the marine nearshore and estuarine environment during the sampling period (July 2017 – September 2019). Numbers above each bar indicate the overall mean CPUE for each habitat.

Overall, significant differences in mean CPUE among life stages in the marine environment (ANOVA: $F_{(4,280)} = 18.23$; $P < 0.001$) and estuarine environment ($F_{(4,825)} = 85.1$; $P = 0.01$) were observed. Significant differences were also observed for each life stage between the marine nearshore and estuarine environments (ANOVA: $F_{(4,1105)} = 20.3$; $P = 0.001$). The mean CPUE of YOY, late juveniles and adults were significantly higher in the estuarine environment (YOY = 77.3 fish per 1000 m^2, late juveniles = 28.0 fish per 1000 m^2 and adult = 57.7 fish per 1000 m^2) compared to the marine nearshore (YOY = 14.6 fish per 1000 m^2, late juveniles = 2.5

fish per 1000 m^2 and adults = 0.6 fish per 1000 m^2) (Tukey's HSD test; $P > 0.05$) (Figure 5.2). Although, the mean CPUE of both postflexion and transformation stages was higher in the estuarine environment (postflexion = 9.7 fish per 1000 m^2 and transformation stages = 16.8 fish per 1000 m^2) than the marine nearshore (postflexion = 2.3 fish per 1000 m^2 and transformation stages = 2.9 fish per 1000 m^2), these differences were not significant (Tukey's HSD test; $P > 0.05$) (Figure 5.2).

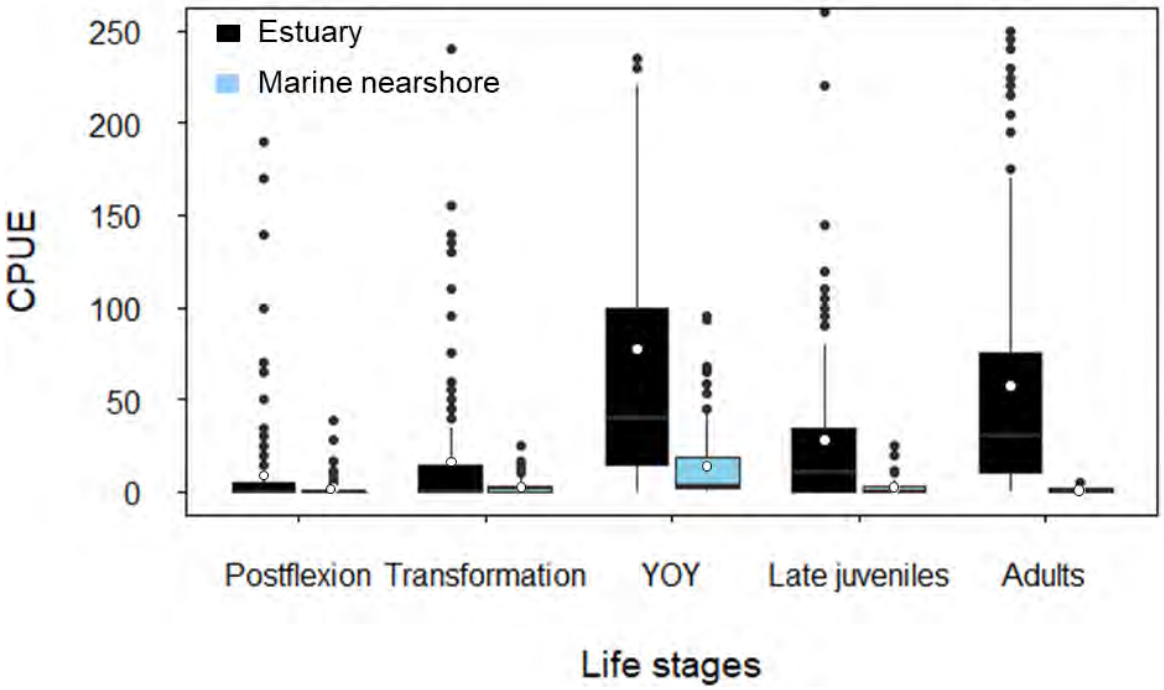

Figure 5.2: Box plots showing the median (thick solid black lines), mean (white circles) and ranges of catch-per-unit-effort (CPUE) (fish per 1000 m^2) of all the life stages recorded in the marine nearshore and estuarine environment during the sampling period (July 2017 – September 2019). Boxes represent the upper and lower limits of the third and first quartiles, and whiskers represent the range. Black dots represent outliers.

5.3.2 Seasonal variation in abundance by life stage

For settlement stage fishes, there were significant differences in mean CPUE among seasons in the estuarine environment (ANOVA: $F_{(3,162)} = 10.7$; $P < 0.001$) with no significant differences in the marine environment (ANOVA: $F_{(3, 53)} = 1.6$; $P = 0.2$). Although the mean CPUE for all the seasons was higher in the estuarine environment than the marine nearshore, these differences were not significant (ANOVA: $F_{(3, 215)} = 2.3$; $P = 0.08$). Higher abundances of the settlement stages were recorded in summer and spring, with the lowest abundances

recorded during winter in both the estuary and marine nearshore. The mean CPUE of settlement stage fishes recorded in spring was siginificantly higher (marine nearshore 7 fish per 1000 m^2 and estuary 51 fish per 1000 m^2) than in autumn and winter in both habitats (Tukey's HSD test; $P < 0.05$) (Figure 5.3a).

For the juvenile stages, a similar trend was observed, with significant differences in mean CPUE observed among seasons in the estuarine environment (ANOVA: $F_{(3, 162)} = 4.2$; $P = 0.01$) but no significant differences were detected in the marine nearshore (ANOVA: $F_{(3, 53)} = 0.8$; $P = 0.51$). There were also no significant differences among seasons between habitats (ANOVA: $F_{(3, 215)} = 0.7$; $P = 0.53$), with mean CPUE for juvenile stage fishes higher in summer in the marine nearshore (24 fish per 1000 m^2) and the estuary (152 fish per 1000 m^2) and to a lesser extent in winter (Figure 5.3b). The Post hoc test showed that summer was significantly different from winter (Tukey's HSD test; $P < 0.05$) in the estuarine environment (Figure 5.3b).

For adults, there were significant differences in mean CPUE among seasons in the estuarine environment (ANOVA: $F_{(3, 162)} = 3.5$; $P = 0.01$) with no significant differences in the marine nearshore (ANOVA: $F_{(3, 53)} = 0.9$; $P = 0.4$). Mean CPUE of adults was also significantly higher in the estuarine environment in all the seasons when compared to the marine nearshore (ANOVA: $F_{(3, 215)} = 3.4$; $P = 0.01$). In the marine nearshore, adults were mostly recorded in winter (1.2 fish per 1000 m^2) and spring (0.8 fish per 1000 m^2). In the estuarine environment, adult stages were significantly (70 fish per 1000 m^2) higher in spring than summer (Tukey's HSD test: $P < 0.05$) (Figure 5.3c).

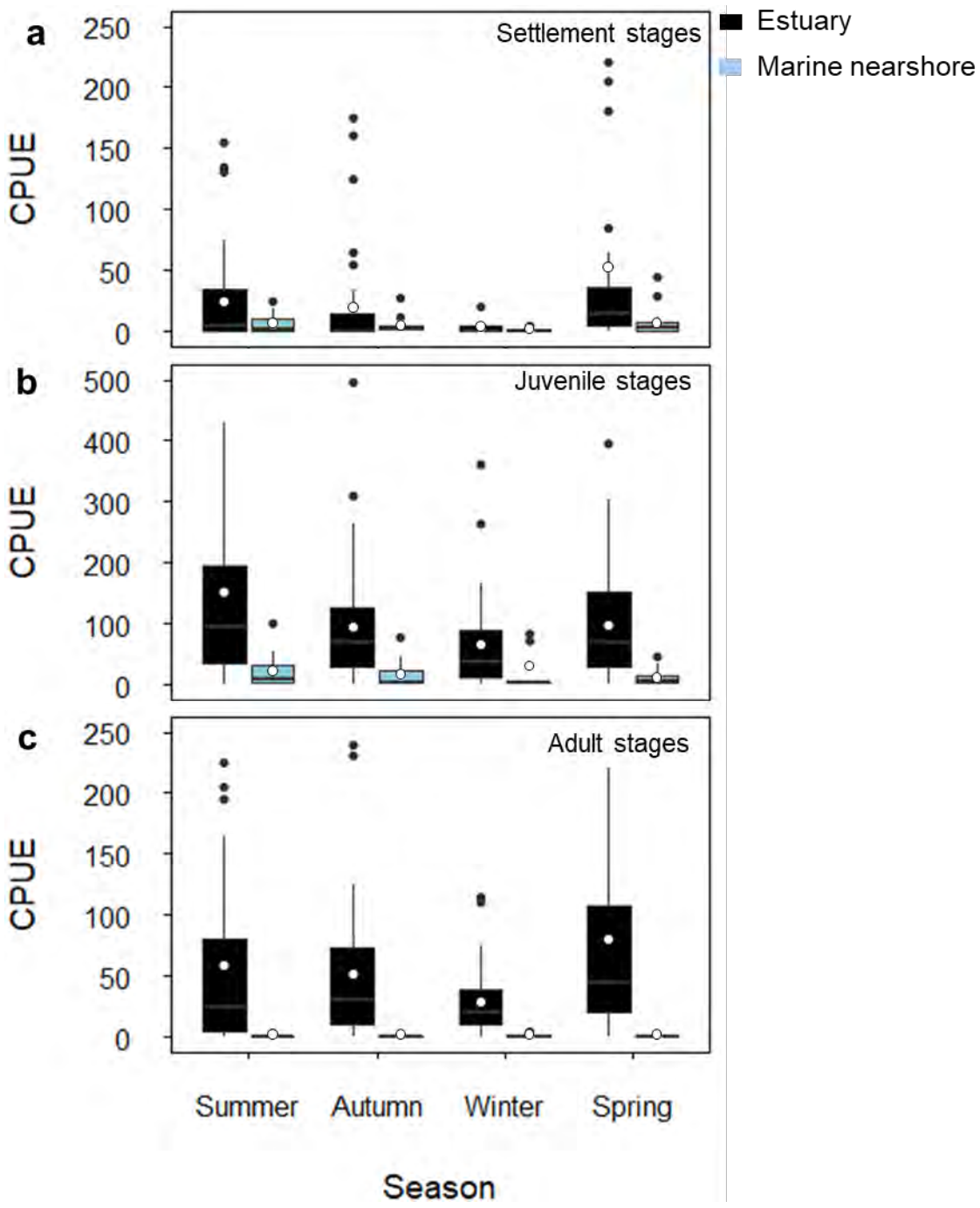

Figure 5.3: Box plots showing the median (thick solid black lines), mean (white circles) and ranges of the catch-per-unit-effort (CPUE) (fish per 1000 m^2) of (a) settlement stages, (b) juveniles and (c) adults recorded in the marine nearshore and the estuary during the sampling period (July 2017 – September 2019). Boxes represent the upper and lower limits of the third and first quartiles, and whiskers represent the range; and thick solid lines the median. Black dots represent outliers.

5.3.3 Species composition in the marine nearshore and estuary by life stage

Settlement stages

The number of species that settled only in the marine nearshore (not in the estuarine environment) was higher (10 species) than in the estuarine environment (six species). Six species settled in both environments comprising 27% of the number of settlement stage species recorded during the study (Figure 5.4). In the marine nearshore, catches of postflexion larvae were numerically dominated by the MS and M species *Sardinops sagax* (MS) (20%) and *Atractoscion aequidens* (M) (17%) and E&M *Caffrogobius saldanha* (22%) (Table 5.2). The transformation stage was numerically dominated by MEO species *Pomadasys olivaceus* (42%) and *Gelichthys feliceps* (22%) (Table 5.2). In the estuarine environment, postflexion larvae were dominated by MED *Rhabdosargus holubi* (60%) and E&F *Glossogobius callidus* (14%) (Table 5.2). The transformation stages were also dominated by *R. holubi* (32%) and *G. callidus* (19%) (Table 5.2).

Juveniles

The number of species that occurred as juveniles only in the estuarine environment (18 species) (43%) was the same as that recorded in the marine nearshore (18 species) (43%). Six species (14% of the 42 species recorded as juveniles) occurred as juveniles in both habitats (Figure 5.4). In the marine nearshore, YOY catches were numerically dominated by MEO *G. feliceps* (49%), *P. olivaceus* (24%) and marine species *Argyrosomus inodorus* (MS) (5%) and *Cynoglossus zanzibarensis* (M) (4%) (Table 5.2). The late juvenile stage was dominated by MEO *G. feliceps* (38%) and the MS *Dasyatis chrysonota* (27%) (Table 5.2). In the estuarine environment, YOY were dominated by MED *R. holubi* (29%) and *Heteromycteris capensis* (16%) (Table 5.2). The late juvenile stage was dominated by E&F *Glossogobius callidus* (I) (38%) and E&M *Caffrogobius gilchristi* (I) (30%) (Table 5.2).

Adults

Only three species were recorded as adults in the marine nearshore (20%), while 10 species (67%) were recorded in the estuarine environment (Figure 5.4). Two species (which comprised 13% of the 15 taxa recorded as adults) were found in both environments (Figure 5.4). In the marine nearshore, marine estuarine-dependent (MED) *Solea turbynei* (47%), marine (M) *C. zanzibarensis* (M) (22%) and MEO *G. feliceps* (13%) numerically dominated the adult life stage (Table 5.2). Marine estuarine-dependent (MED) *S. turbynei* (28%) also dominated this

life stage in the estuarine environment followed by E&M *Psammogobius knysnaensis* (I) (26%) and SE *Gilchristella aestuaria* (22%) (Table 5.2).

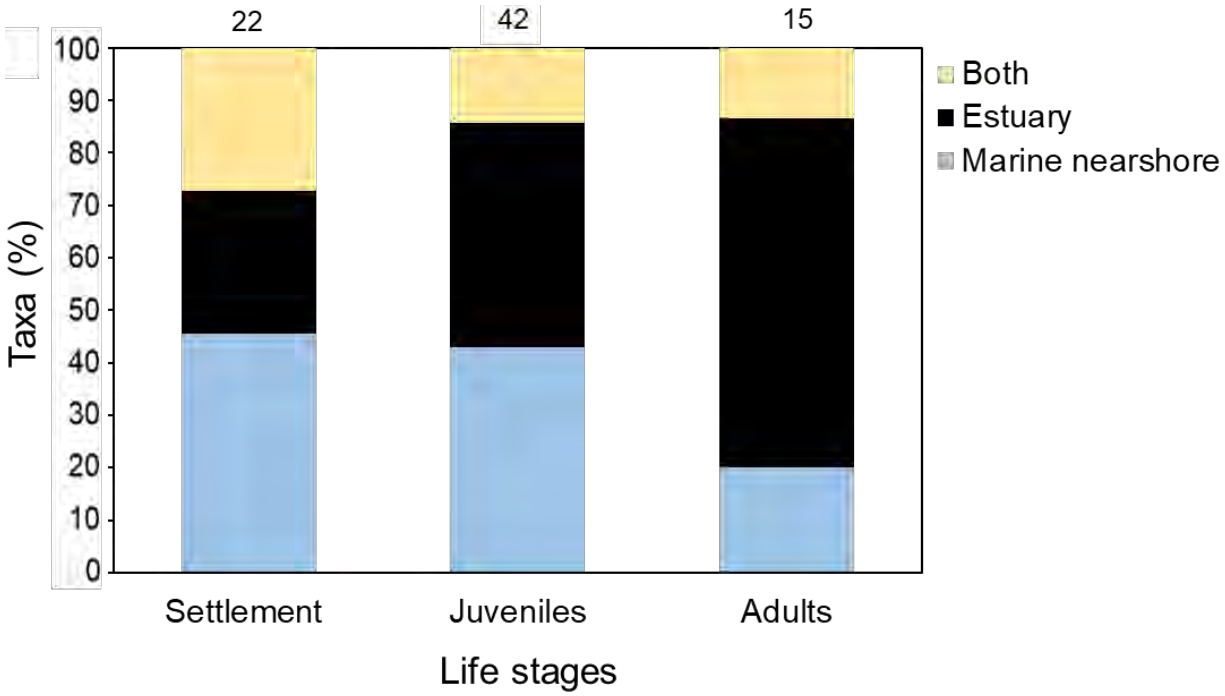

Figure 5.4: Percentage contribution of species (%) for the settlement stages, juveniles and adults to the total taxa caught in the marine nearshore, estuary and in both habitats during the sampling period (between July 2017 and September 2019). Numbers above each bar indicate the total number of species caught for each life stage.

Table 5.2: Total number of individuals, mean catch-per-unit effort (CPUE) (fish per 1000 m^2) and the percentage contribution (%) of each species to the total catch of each life stage in the marine nearshore and estuarine environment during the sampling period (July 2017 – September 2019). Whitfield estuarine association category (Whitfield 1994, 2019) and proposed amendments to the Whitfield (2019) estuary association category of some species. Estuarine association guild using the international classification scheme of Potter et al. (2015). Species caught in the marine nearshore and estuarine environment are shaded in blue. Grey shading shows species only caught in one environment (nearshore marine or estuarine).

Family	Species	Marine nearshore					Estuary					Estuary Category (& proposed category)	Fish guild
		Post-flexion	Trans-formation	YOY	Late juveniles	Adults	Post-flexion	Trans-formation	YOY	Late juveniles	Adults		
Ariidae	*Galeichthys feliceps*	-	22; 0.6 (22)	249;7.3 (49)	34; 0.9(38)	3; 0.1(13)		39; 1.2(7)	17; 0.5(0.7)	5; 0.2(0.5)	4; 0.1(0.2)	IIb (IIc)	MEO
Atherinidae	*Atherina breviceps*	-	-	-	-	-	-	-			121; 3.6(6)	I	E&M
Blenniidae	*Omobranchus woodi*	-	-	-	-	-	-	-	1; 0.03(0.04)	2; 0.1(0.2)	1; 0.03(0.1)	I	E&M
	Parablennius cornutus	-	-	1; 0.03(0.2)			-	-	-		-	M	M
Carangidae	*Trachurus trachurus*	-	1; 0.03(1)	1; 0.03(0.2)	2; 0.1(2)	-	-	-	-		-	III	MS
Clupeidae	*Gilchristella aestuaria*	-	-	-	-		-	-	97; 2.9(3.8)	27; 0.8(2.8)	446; 13.4(22)	I	SE
	Sardinops sagax	17; 0.5(20)	-	-	-	-	-	-	-	-	-	III	MS
Cynoglossidae	*Cynoglossus capensis*	-	-	3; 0.1(0.6)	2; 0.1(2)	-	-	-	-	-	-	M	M
	Cynoglossus zanzibarensis	-	7; 0.2(7)	19; 0.6(4)	9; 0.2(10)	5; 0.2(22)	-	-	-	-	-	M	M
Dasyatidae	*Dasyatis chrysonota*	-		3; 0.1(0.6)	24; 0.7(27)	2; 0.1(9)	-	-	-	-	-	III	MS
Elopidae	*Elops machnata*		-	-	-	-	-	-	11; 0.3(0.4)	-	-	IIa	MED
Engraulidae	*Engraulis capensis*	6; 0.2(7)	-	-	-	-	-	-	-	-	-	III	MS
Gobiidae	*Caffrogobius agulhensis*	-	-	1; 0.03(0.2)	--	-	-	-	-	-	-	III	MS

	Caffrogobius gilchristi	-	-	-	-	-	18; 0.5(6)	10; 0.3(2)	351; 10.6(14)	284; 8.6(30)	297; 8.9(15)	I	E&M
	Caffrogobius nudiceps	-	-	-				3; 0.1(0.5)	3; 0.1(0.1)	3; 0.1(0.3)	12; 0.4(0.6)	I	E&M
	Caffrogobius saldanha	18; 0.5(22)	-	-	-	-	2; 0.1(0.6)	-	-	-		I	E&M
	Glossogobius callidus	-	-	-	-	-	47; 1.4(14)	107; 3.2(19)	202; 6.1(7.9)	353; 10.6(38)	38; 1.1(1.9)	I	E&F
	Psammogobius knysnaensis	-	-	-	-	-	2; 0.1(0.6)	56; 1.7(10)	166; 5.0(6.5)	41; 1.2(4.4)	529; 15.9(26)	I	E&M
Gymnuridae	*Gymnura natalensis*	-	-	2; 0.1(0.4)	2; 0.1(2)	-	-	-	--	-	-	M	M
Haemulidae	*Pomadasys commersonnii*	-	-	-	-	-	-	21; 0.6(3.7)	113; 3.4(4.4)	75; 2.3(8)	2; 0.1(0.1)	IIa	MED
	Pomadasys olivaceus	2; 0.1(2)	43; 1.3(43)	124; 3.6(24)	11; 0.3(12.5)	1; 0.03(4)	-	8; 0.2(1.4)	19; 0.6(0.7)	-	-	IIc	MEO
Monodactylidae	*Monodactylus falciformis*	-	-	-	-	-	-	-	1; 0.03(0.04)	1; 0.03(0.1)	-	IIa	MED
Mugilidae	*Chelon dumerili*	-	-	-	-	-	-	-	1; 0.03(0.04)	1; 0.03(0.1)	-	IIa	MED
	Pseudomyxus capensis	-	-	-	-	-	-	-	1; 0.03(0.04)	-	-	IIa	MED
Myliobatidae	*Myliobatis aquila*	-	-	2; 0.1(0.4)	-	-	-	-		-		IIc	MEO
Ostraciidae	*Ostracion cubicus*	-	-	-	-	-	-	-	1; 0.03(0.04)	-	-	III	MS
Platycephalidae	*Platycephalus indicus*	-	-	-	-	-	-		3; 0.1(0.1)	5; 0.2(0.5)	8; 0.2(0.4)	IIc	MEO
Rajidae	*Raja clavata*	-	-	1; 0.03(0.2)	-	-	-	-	-	-	-	M	M
	Raja miraletus	-	-	2; 0.1(0.4)	-	-	-	-	-	-	-	M	M
Rhinobatidae	*Acroteriobatus annulatus*	-	-	2; 0.1(0.4)	-	-	-	-	-	-	--	III	MS
Sciaenidae	*Argyrosomus japonicus*	-	-	-	--	-	-	4; 0.1(0.7)	6; 0.2(0.2)	5; 0.2(0.5)	-	IIa	MED
	Atractoscion aequidens	14; 0.4(17)	-	-	-	-	-	-	-	-	-	M	M
	Umbrina canariensis	-	2; 0.1(2)	21; 0.6(4.2)		-	-	-	-	-	-	M	M
	Argyrosomus inodorus	13; 0.4(16	6; 0.2(6)	24; 0.7(5)	3; 0.1(3.4)	-	-	-	-	-	-	M	M

Soleidae	*Heteromycteris capensis*	-	-	3; 0.1(0.6)	1; 0.03(1.1)	-	12; 0.4(4)	36; 1.1(6.4)	409; 12.3(16)	2; 0.1(0.2)	-	IIa	MED
	Solea turbynei	-	-	-	-	11(47%)	25; 0.8(8)	57; 1.7(10.2)	152; 4.6(5.9)	113; 3.4(12)	577; 17.4(28)	IIa	MED
Sparidae	*Cheimerius nufar*	-	-	1; 0.03(0.2)	-				-			III	MS
	Diplodus capensis	5; 0.2(6)	2; 0.1(2)	-	-	-	1; 0.03(0.3)	7; 0.2(1.3)	8; 0.2(0.3)	-	-	IIc	MEO
	Lithognathus lithognathus	-	-	-	-	-	-	-	14; 0.4(0.5)	-	-	IIa	MED
	Rhabdosargus globiceps	-	-	18; 0.5(4)	-	-	17; 0.5(5.3)	22; 0.7(3.9)	224; 6.7(8.7)	-	-	IIc	MEO
	Rhabdosargus holubi	3; 0.1(4)	4; 0.1(4)	1; 0.03(0.2)	-	-	198; 5.9(60)	182; 5.5(32)	753; 22.7(29)	14; 0.4(1.5)	2; 0.1(0.1)	IIa	MED
	Rhabdosargus thorpei	-	-	-	-	-	-	-	1; 0.03(0.03)	-	-	IIb	MEO
	Sarpa salpa	-	-	1; 0.03(0.2)	-	-	-	-	-	-	-	IIc	MEO
	Spondyliosoma emarginatum	2; 0.1(2)	7; 0.2(7)	13; 0.4(3)	-	-	-	7; 0.2(1.3)	8; 0.2(0.3)	-	-	IIb	MEO
Syngnathidae	*Syngnathus temminckii*	-	-	-	-	-	-	-	-	-	1; 0.03(0.04)	I	E&M
Tetraodontinae	*Geneion honckenii*	-	2; 0.1(2)	2; 0.1(0.4)	-	3; 0.1(4)	-	-	-	-	-	IIc	MEO
Torpedinidae	*Torpedo fuscomaculata*	-	-	-	-	-	-	-	1; 0.03(0.04)	1; 0.03(0.1)	-	IIc	MEO
Triglidae	*Chelidonichthys kumu*	-	4; 0.1(4)	5; 0.2(1)	-	-	-	-	-	-	-	III	MS
	Unidentified	3; 0.1(4)	-	3; 0.1(0.6)	-	-		-	6; 0.1(0.2)	-	-		

5.3.4. Spatial trends in the abundance of dominant marine (M), marine estuarine-opportunist (MEO), marine estuarine-dependent (MED) and estuarine (E&M and E&F) species (by life stage) across the estuary-ocean gradient

Marine species (M)

In the marine nearshore, settlement and juvenile stages of the marine species (M) *C. zanzibarensis* and *A. inodorus* were only caught in the marine environment, more specifically at sites closest to the mouth of the two estuaries and harbours, with the higher mean abundance of *C. zanzibarensis* juveniles recorded adjacent to the Swartkops Estuary mouth (1.5 – 2.0 fish per 1000 m^2) (Figure 5.5). *Argyrosomus inodorus* juveniles were caught near the Sundays and Swartkops estuaries with higher mean abundance recorded near the Papenkuils outlet (2.1 – 3.0 fish per 1000 m^2). Adult *C. zanzibarensis* (0.2 – 0.5 fish per 1000 m^2) were caught near the Swartkops Estuary mouth, Papenkuils outlet and Port Elizabeth Harbour, and no adult *A. inodorus* were caught during the study (Figure 5.5).

Figure 5.5: Maps showing mean catch-per-unit-effort (CPUE) (fish per 1000 m^2) of *Cynoglossus zanzibarensis* and *Argyrosomus inodorus* (marine species) settlement stages, juveniles and adults recorded in each site in the marine nearshore, Sundays and Swartkops estuaries during the sampling period (July 2017 – September 2019).

Marine estuarine-opportunists (MEO)

The highest mean abundance of *P. olivaceus* (MEO) settlement stages was recorded in the marine nearshore at sites closest to Port of Ngqura and Sundays Estuary mouths (2.1 – 3.4 fish per 1000 m^2) (Figure 5.6). Although the abundance of juvenile *P. olivaceus* was highest near the Port of Ngqura (10.0 fish per 1000 m^2), juveniles were abundant at almost all sites in the marine nearshore and the lower reaches of the Sundays Estuary (1.4 – 7.3 fish per 1000 m^2). Very few (0.2 fish per 1000 m^2) adult *P. olivaceus* were recorded in the marine nearshore and these were caught near the Sundays Estuary mouth. *Galeichthys feliceps* (MEO) settled in higher numbers (1.5 – 3.0 fish per 1000 m^2) near the Papenkuils outlet in the marine nearshore and in the lower and middle reaches of the Sundays Estuary (1.5 – 13.0 fish per 1000 m^2). High numbers of juveniles (31.9 fish per 1000 m^2) were also recorded near the Papenkuils Outlet. Adult *G. feliceps* were caught near the Sundays Estuary mouth and in the lower reaches of the Sundays Estuary (0.4 – 1.3 fish per 1000 m^2) (Figure 5.6).

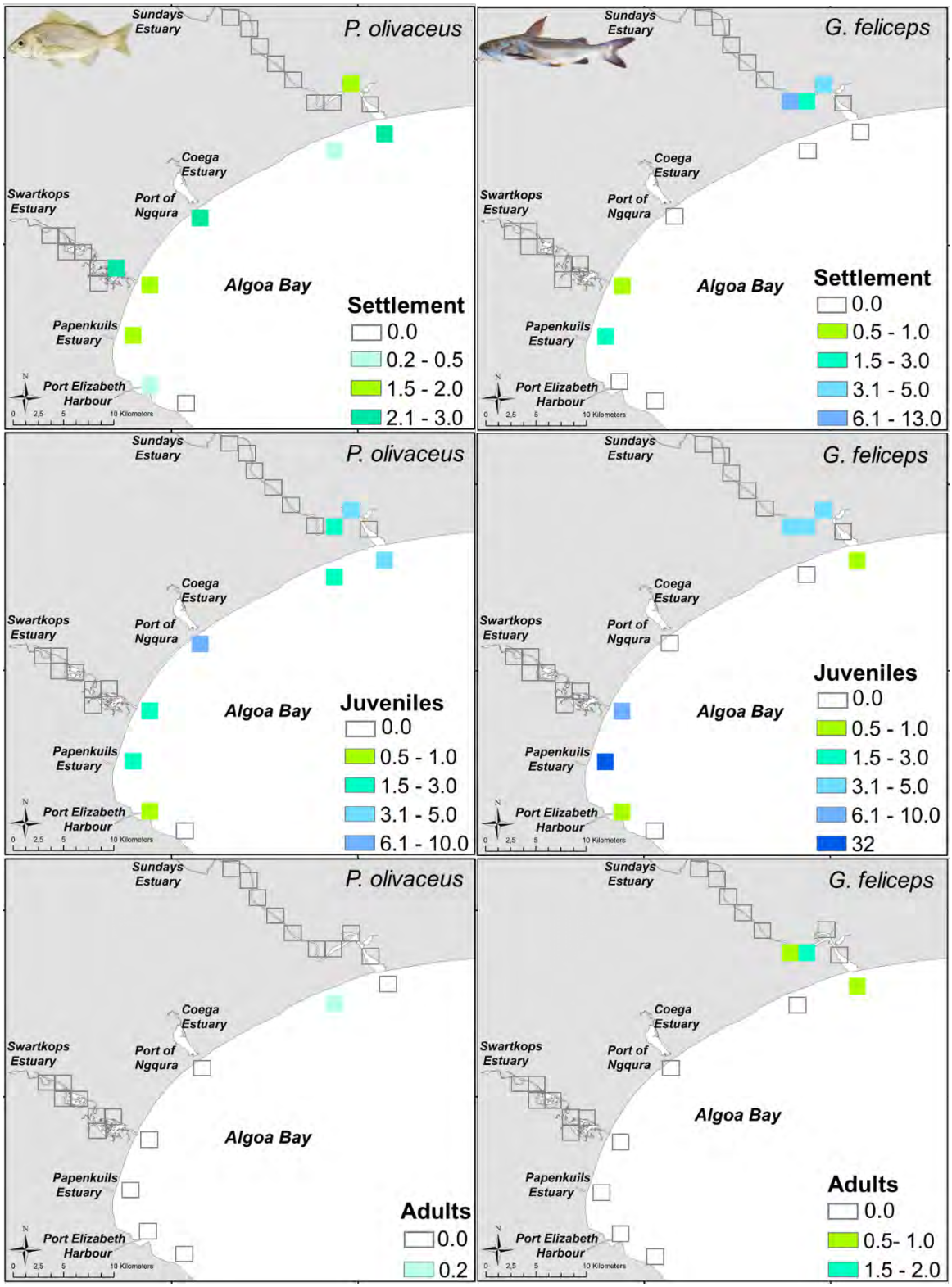

Figure 5.6: Maps showing mean catch-per-unit-effort (CPUE) (fish per 1000 m^2) of the *Pomadasys olivaceus* and *Galeichthys feliceps* (marine estuarine-opportunists) settlement stages, juveniles and adults recorded in each site in the marine nearshore, Sundays and Swartkops estuaries during the sampling period (July 2017 – September 2019).

Marine Estuarine-Dependent (MED)

Rhabdosargus holubi (IIa) settlement and juvenile stages were most abundant in the upper reaches of the Swartkops Estuary and the lower reaches of the Sundays Estuary, with few settlement stage individuals caught in the marine nearshore in close proximity to the Sundays Estuary (1.5 – 2.0 fish per 1000 m^2). *Rhabdosargus holubi* adults were only recorded in the lower reaches of the Sundays Estuary (Figure 5.7a). Similar patterns were found for the settlement and juvenile stages of *S. turbynei* (IIa) although no settlement or juvenile stages were recorded in the marine nearshore (Figure 5.7a). *Solea turbynei* adults were caught in both environments, throughout the estuaries, with the highest CPUE (65 fish per 1000 m^2) recorded in the lower reaches of the Sundays Estuary (Figure 5.7a). *Heteromycteris capensis* (IIa) settlement stages were only recorded in the lower and middle reaches of the Swartkops and Sundays estuaries (Figure 5.7b). *Heteromycteris capensis* juveniles were also caught only in the lower and middle reaches with highest catches (40.0 – 91.5 fish per 1000 m^2) recorded in the lower reaches and few individuals (0.5 – 1.0 fish per 1000 m^2) were caught in the marine nearshore (near the Swartkops Estuary mouth and the Port of Ngqura) (Figure 5.7b).

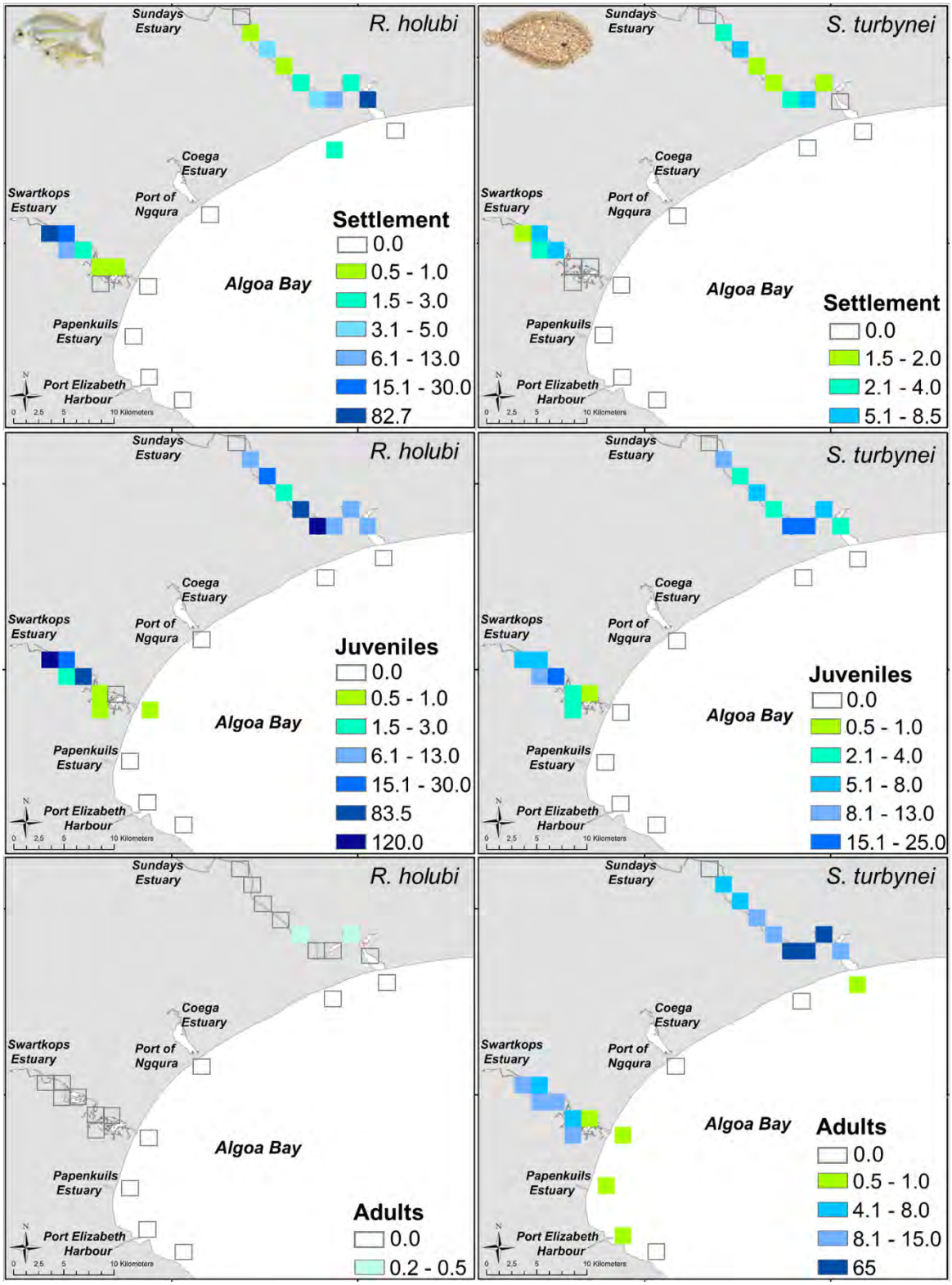

Figure 5.7a: Maps showing mean catch-per-unit-effort (CPUE) (fish per 1000 m^2) of *Rhabdosargus holubi* and *Solea turbynei* (marine estuarine-dependent) settlement stages, juveniles and adults recorded in each site in the marine nearshore, Sundays and Swartkops estuaries during the sampling period (July 2017 – September 2019).

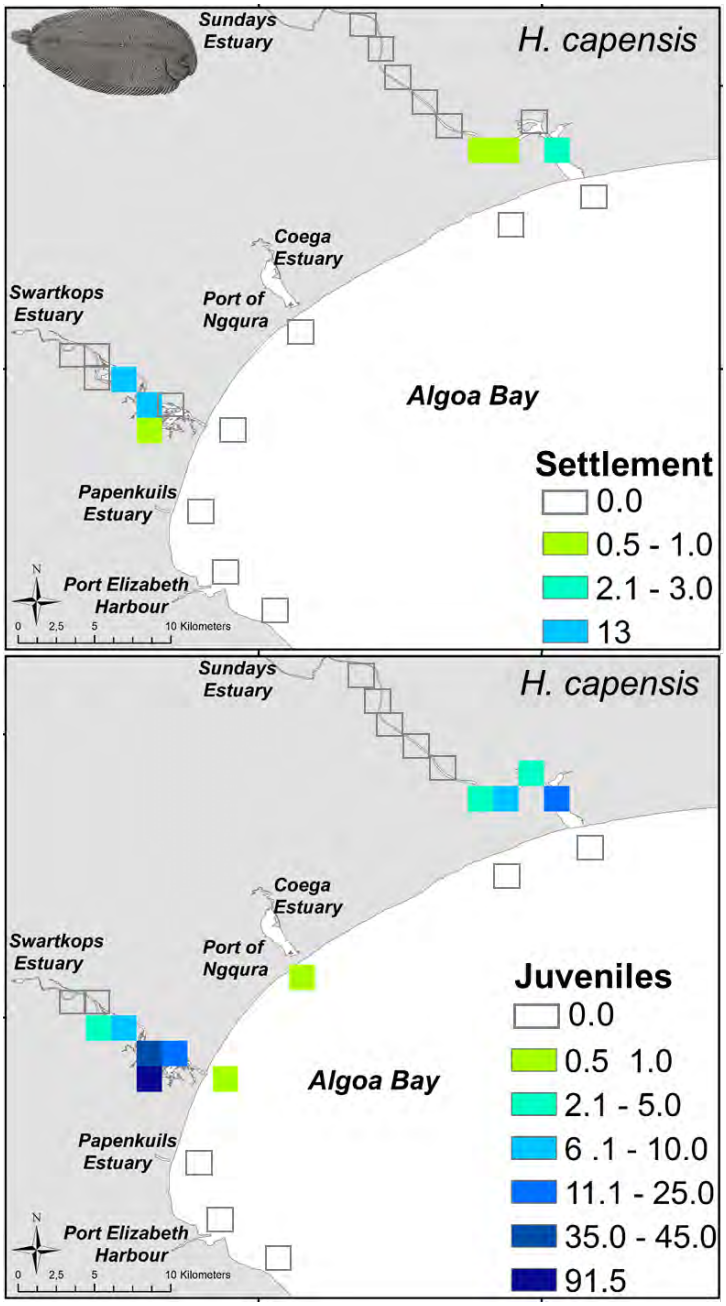

Figure 5.7b: Maps showing the mean catch-per-unit-effort (CPUE) (fish per 1000 m^2) of *Heteromycteris capensis* (marine estuarine-dependent) settlement stages, juveniles and adults recorded in each site in the Sundays and Swartkops estuaries during the sampling period (July 2017 – September 2019).

Estuarine and marine and estuarine and freshwater species (E&M, E&F)

All three abundant estuarine resident species from the Gobiidae family; *P. knysnaensis* (E&M), *C. gilchristi* (E&M) and *G. callidus* (E&F) were only caught in the two estuaries. The settlement and juvenile stages of *P. knysnaensis* were more abundant in the middle and lower reaches of the Swartkops Estuary and the lower reaches (mouth region) of the Sundays Estuary (11.1 – 55.5 fish per 1000 m^2). Although the adult stage of *P. knysnaensis* was distributed throughout both estuaries, the highest abundance (75.0 fish per 1000 m^2) was observed in the lower reaches (Figure 5.8a). The settlement stage of *C. gilchristi* were predominantly found in the middle reaches of the Swartkops Estuary and the lower reaches of the Sundays Estuary (2.1 – 27.0 fish per 1000 m^2). The juvenile and adult stages of *C. gilchristi* were most abundant in the upper reaches of the Swartkops Estuary and the lower reaches of the Sundays Estuary (93 fish per 1000 m^2) (Figure 5.8a). The settlement (10.0 – 27.0 fish per 1000 m^2) and juvenile (21 – 107.3 fish per 1000 m^2) stages of *G. callidus* were abundant in the upper reaches of both estuaries. Adult *G. callidus* were recorded in lower numbers (3.0 – 8.0 fish per 1000 m^2) in both estuaries, with adults only recorded in the upper reaches of the Swartkops Estuary and throughout the Sundays Estuary (Figure 5.8b).

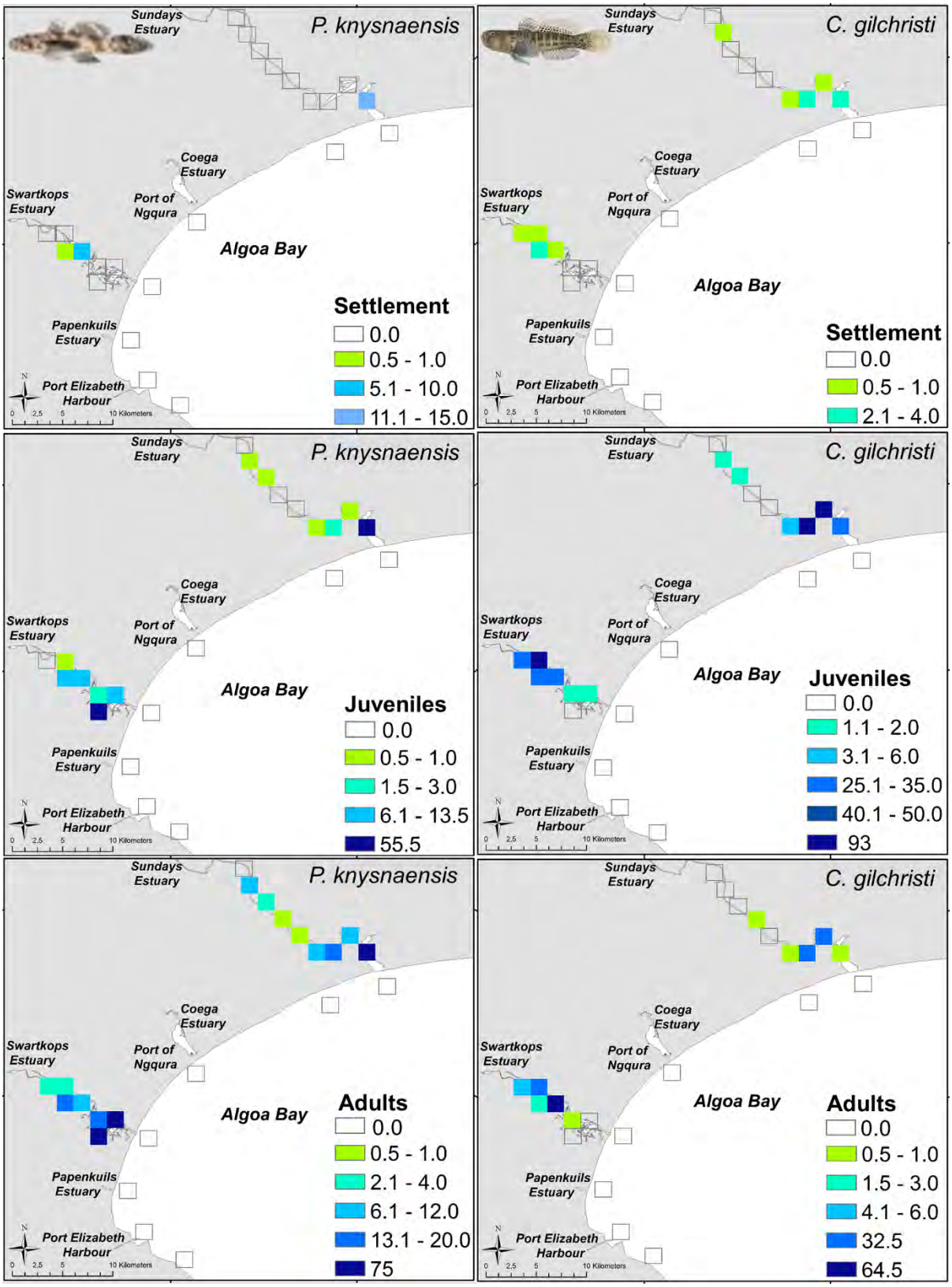

Figure 5.8a: Maps showing mean catch-per-unit-effort (CPUE) (fish per 1000 m^2) of *Psammogobius knysnaensis* (estuarine and marine) and *Caffrogobius gilchristi* (estuarine and marine) settlement stages, juveniles and adults recorded in each site in the Sundays and Swartkops estuaries during the sampling period (July 2017 – September 2019).

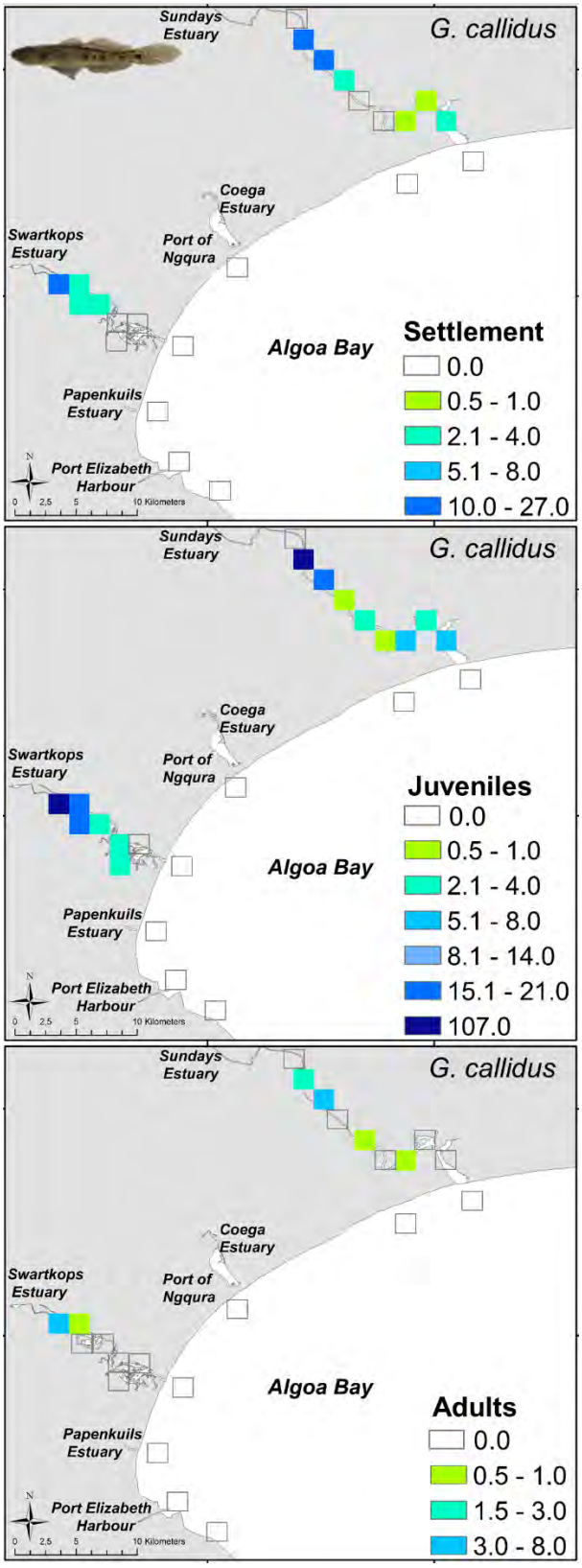

Figure 5.8b: Maps showing mean catch-per-unit-effort (CPUE) (fish per 1000 m^2) of *Glossogobius callidus* (estuarine and freshwater) settlement stages, juveniles and adults recorded in each site in the Sundays and Swartkops estuaries during the sampling period (July 2017 – September 2019).

5.4 Discussion

In this study, estuarine (Sundays and Swartkops) and marine nearshore (Algoa Bay) environments were dominated by early life history stages (larvae to juveniles) of fish, but the overall abundance of settlement stage individuals and juveniles was five to six times higher in the estuarine environment. These results suggest that although both environments provide important settlement and nursery habitat for demersal fish species, soft-bottom benthic estuarine environments are far more productive nursery areas for demersal species when compared to soft-bottom benthic nearshore marine environments. Settlement and nursery habitats also differed by estuarine-association, where the nearshore marine environment dominated in terms of CPUE by marine and marine estuarine-opportunists, with core settlement and nursery areas for these species located close to estuary mouths, freshwater outlets and harbours. In contrast, the estuarine environment was dominated by marine estuarine-dependent and estuarine species (E&M), with the upper reaches of the Swartkops Estuary and the lower reaches of the Sundays Estuary identified as important settlement and nursery areas for these species.

The fact that both environments were collectively dominated by settlement and juvenile stages indicates that both environments provide settlement and nursery habitat for the early life history stages of fish (Able et al. 2006; Guerreiro et al. 2021). Martino and Able (2003) found that YOY juveniles dominated fish assemblages from the low salinity zone of the Mullica River–Great Bay Estuary to the marine nearshore in southern New Jersey (USA). Similarly, more than 50% of the demersal fishes in both the Tagus Estuary and adjacent nearshore waters (Portugal) were juveniles, which indicates that both the marine nearshore and the estuary function as nursery areas to some extent (Prista et al. 2003).

The overall abundance of YOY, juveniles and adults was significantly higher in the estuarine environment than the marine nearshore. This highlights the high productivity of the estuarine environment as well as the importance of estuarine nursery areas for demersal species in South Africa, where the exposed coastline is characterised by high-energy wave action and strong ocean currents (McLachlan et al. 1981; Beckley 1985; Potter et al. 1990). The high productivity of estuaries has often been identified as a primary reason why larval and juvenile fish are attracted to these areas in such large numbers (Whitfield 1999). For example, Miró et al. (2020), who sampled early life stages of fishes (larval and transformation stages) in the permanently

open Guadiana, Odiel-Tinto, Guadalquivir and Cadiz estuaries and adjacent marine nearshore environment in the Gulf of Cadiz (south-west Iberian Peninsula) using a plankton net, attributed the significantly higher abundances of early life stages in the Guadalquivir Estuary compared to the nearshore to the higher productivity of estuaries. Similarly, Valesini et al. (1997) sampled littoral fish assemblages in the Blackwood River Estuary and nearshore waters of Flinders Bay in temperate Australia using a seine net and found significantly higher abundance of fishes in the estuary compared to the nearshore sites and linked this to the high productivity of the estuary, which provides greater sources of food and/or protection than the nearby moderately-protected marine nearshore waters. Similarly, Potter et al. (2000) collating information from seine and gill net surveys of the Leschenaalt Estuary and the adjacent marine nearshore embayment Koombana Bay in temperate Australia and found that overall the abundance of fish recorded in the estuary was 12 times higher in the estuary than the nearshore and this was attributed to the higher productivity and protection provided by estuarine systems.

Although both habitats were dominated by early life history stages, different species dominated these early life history stages in each environment. The marine nearshore was dominated by the settlement and juvenile stages of marine estuarine-opportunists *G. feliceps* and *P. olivaceus* and marine species *C. zanzibarensis* and *A. inodorus.* While the estuarine environment was dominated by the settlement and juvenile stages of marine estuarine-dependent *R. holubi*, *S turbynei* and *H. capensis* and estuarine resident species (which includes estuarine and freshwater and estuarine and marine species) *C. gilchristi, G. callidus* and *P. knysnaensis*. Similarly, Guerreiro et al. (2021) studied fish assemblages (with WP3 ring net) in the Mondego Estuary and the adjacent exposed Atlantic coastal areas on the northwest coast of Portugal and found that the estuarine fish assemblage was dominated by juvenile estuarine species, while marine nearshore areas were dominated by juvenile marine species. It was concluded that, although both environments were dominated by early life stages (highlighting the nursery role of these habitats), the Mondego Estuary and the adjacent marine nearshore environments provide nursery areas for different species.

The results from the present study contrast with findings from temperate North America, where the embayments associated with many large American estuaries provide protection not only for resident estuarine fish species but also for a wide range of marine species (Weinstein 1985). Able et al. (2006) compared settlement and juvenile stages (collected fish using beam trawl and plankton nets) of demersal species across an estuary-ocean ecotone (Great Bay-Little Egg

Harbour estuary and adjacent inner continental shelf) in southern New Jersey (USA) and found that many species commonly occurred in both habitats (estuary and marine nearshore) as settlement stages and juveniles indicating a high degree of overlap between these two environments.

In the present study, of those species that occurred in both habitats as settlement (six species) and juvenile stages (six species), most marine estuarine-opportunist species (three species) were recorded in higher abundance in these early stages in the marine nearshore environment compared to the estuaries. For example, settlement and juvenile *G. feliceps* and *P. olivaceus* occurred in higher abundances in the marine nearshore environment than in the estuaries. *Pomadasys olivaceus* and *G. feliceps* have been shown to be abundant in the shallow marine environment of Algoa Bay (Lasiak 1983; Buxton et al. 1984). Only a few juvenile *G. feliceps* were recorded in the lower reaches of the estuaries (in the Sundays Estuary only). *Galeichthys feliceps* is currently categorised by Whitfield (1994, 2019) as a marine species whose juveniles occur mainly in estuaries but are also common at sea (IIb). In light of the findings from my study, it is recommended that the estuary-association category for this species change to IIc (in which juveniles sometimes occur in estuaries but are more abundant at sea). *Pomadasys olivaceus* was previously categorised by Whitfield (1998) as a marine straggler (III) which occurs in the lower reaches of estuaries in very low numbers and are not dependent on these systems. Whitfield (2019) later categorised this species as IIc, where juveniles sometimes occur in estuaries but are more abundant at sea. The findings of the present study confirm the recent Whitfield (2019) reclassification for this species.

Of the species recorded in both estuaries and the marine nearshore (Chapter Four) most are not recorded in both habitats at all life stages. For example, settlement and juvenile stages of the flatfish, *S. turbynei* and *H. capensis* were recorded only in the estuaries, while adults were caught in both habitats. The larvae of *S. turbynei* have been shown to be abundant in the nearshore and surf zone of Algoa Bay mostly during summer (Strydom et al. 2015). The settlement and juvenile stages of both species are abundant in estuaries (Strydom et al. 2015; Richardson et al. 2006; Vorwerk et al. 2008, Nodo et al. 2017, 2018). *Solea turbynei* and *H. capensis* were previously categorised as marine estuarine-opportunists (IIb) in the Whitfield (1998) classification. They were later reclassified as marine estuarine-dependent species (IIa) by Whitfield (2019). The findings from the present study (settlement and early juveniles only found in estuaries) confirm this reclassification.

Species that were only recorded in estuaries (across all life stages) included the three gobies, *C. gilchristi, G. callidus* and *P. knysnaensis,* which are abundant in South African estuaries as larvae, juveniles and adults (Whitfield 1998, 2019). Early-life stages of these gobies feed on zooplankton in estuaries, which include copepods, with juveniles and adults feeding on amphipods, isopods and insect larvae (Whitfield 1998). The distribution and high abundance of these species in estuaries is also linked to sediment particle size, with *C. gilchrist* and *G. callidus* associated with muddy bottoms and *P. knysnaensis* associated with sandy bottoms (Chapter Four) as well high turbidity, which provides shelter and protection against predation (Blaber 1980; Whitfield 1998).

Spatial analysis of the abundance of dominant species across the estuary-ocean gradient identified different settlement and juvenile hotspots (essential fish habitats with higher density Katara et al. 2021) in the two estuaries. In the Sundays Estuary the lower reaches are important settlement and nursery areas for marine estuarine-dependent as well as estuarine species (*R. holubi*, *S. turbynei*, *C. gilchristi*, *P. knysnaensis* and *G. callidus*). In contrast, the upper reaches of the Swartkops Estuary were identified as important nursery and settlement areas for the same species. Searching for a suitable settlement habitat after the completion of the pelagic larval stage represents a significant challenge to settlement-stage larvae (Grol et al. 2011; Nagelkerken et al. 2015), as such during this stage fish often occupy the first suitable area they come across when entering nursery habitats from the open ocean (Grol et al. 2011; Nagelkerken et al. 2015). It is therefore imperative to identify settlement habitats as this is the critical stage where most important morphological, physiological and behavioural transitions occur (Nagelkerken et al. 2015). Furthermore, as this stage is associated with high mortalities (Almany and Webster 2005), understanding how and where settlement occurs is very important for the management of coastal fishes.

In the marine nearshore, sites closest to estuary mouths, harbours and outlets were identified as important settlement and juvenile hotspots for marine species such as *C. zanzibarensis* and *A. inodorus* (which were only caught in the marine nearshore) and marine estuarine-opportunists *P. olivaceus* and *G. feliceps*. The shallow (0 – 9 m) soft substratum areas of Algoa Bay have been identified as a major nursery area for the important fishery species *A. inodorus* (Smale 1984; Wallace et al. 1984a; Beckley 1986). Juveniles of the commercially important *C. zanzibarensis* are also known to be abundant in the nearshore habitats of Algoa Bay and are among the most abundant species along the Cape south coast (Badenhorst and Smale 1991)

inhabiting areas of coarse and sandy sediments (Booth and Walmsley-Hart 2000). The distribution and abundance of *A. inodorus* in the marine nearshore was associated with high silt/mud content (Chapter Four), which was higher in sites closer to the estuary mouths and outlets.

The greatest abundance of settlement and juvenile stages in both habitats occurred during summer and spring and can be attributed to the recruitment period of larval and juvenile marine species into nursery areas in South Africa (Harrison and Whitfield 1995; Whitfield 1998). Spawning for most marine species occurs between late winter and summer (Whitfield 1998) and an increase in water temperatures and food availability during spring could be related to the higher abundance of early life history stage fishes observed during spring and summer (Whitfield 1994).

Although a lot of research has been done on the fish assemblages of estuaries and the nearshore, this is the first study in South Africa to have used the same gear to sample both environments at the same time. This study shows the importance of concurrently sampling habitats in marine nearshore and estuarine environments which allows for a better understanding of the degree of estuarine dependency of marine fish as well as an understanding of the nursery function of coastal habitats. Access to various habitats is crucial for many species during different life stages and during ontogenetic shifts (Gillanders et al. 2003; Sheaves 2009). These results have shown that although some marine fishes use both habitats as nursery areas, each environment is characterised by fairly distinct fish assemblages, with both dominated by early-life stages emphasising the nursery function of both environments.

CHAPTER SIX

Response of demersal fishes to low dissolved oxygen events in two heavily polluted estuaries

6.1 Introduction

Coastal marine systems may be particularly vulnerable to anthropogenic stressors such as changes in climate, land-based pollution and low dissolved oxygen, all of which lead to environmental degradation and have an imapct on valuable ecosystem services (van Niekerk and Turpie, 2012). Pollution from excessive nutrient enrichment, increasing metals and persistent organic pollutants (POPs), as well as climate change, have all contributed to severe ecological deterioration in the coastal zone (Lu et al. 2018). Furthermore, pollution from human settlements as well as agricultural and industrial activities is a major cause of deteriorating water quality in estuaries (Freeman et al. 2019). An increase in nutrient loading from these inputs leads to eutrophication (Adams et al. 2019), defined by Nixon (1995) as "an increase in the rate of supply of organic matter to an ecosystem". Nutrient (mainly nitrogen and phosphorus) over-enrichment has negative effects on the ecological functioning of estuaries and can result in increased macro and microalgal biomass, harmful algal blooms (HABs), low dissolved oxygen and hypoxia. Ultimately, this may lead to changes in fish community composition and fish kills (Bricker et al. 2003; Lemley et al. 2016).

Low dissolved oxygen (DO) in eutrophic estuaries and coastal waters typically occurs during warmer months (summer), when stratification of the water column inhibits reaeration of the bottom waters (Breitburg et al. 1997; Rabalais et al. 2001; Conley et al. 2009). As water temperature increases during summer, the amount of oxygen that can dissolve into water is also reduced and this can increase the metabolic requirements of aquatic organisms (Breitburg 2002). These conditions (low DO and increased temperatures) may result in loss of suitable habitat (Coutant 1987; Rabalais et al. 2010). Habitat loss due to low DO results in a decline in both aquatic species richness and abundance as well as distribution shifts in estuaries (Pihl et al. 1991; Craig 2012; Hallet et al. 2019).

In estuaries, the impact of low DO on species richness, abundance and distribution is particularly apparent for demersal and benthic fishes, as low DO is mostly limited to the bottom waters (e.g. McClatchie et al. 2010). As DO concentration decreases below 4 mg/l in bottom

waters, benthic or demersal species exhibit stress behaviour and some species move away from the affected areas (Buchheister et al. 2013). While some species may avoid low DO by migrating away from the affected areas, there are species-specific differences in the level of DO that causes this emigration (e.g. Pihl et al. 1991, 1992; Breitburg 1992; Eby and Crowder 2002; Craig 2012). Dissolved oxygen thresholds that induce sub-lethal effects range from 2.0 to 4.5 mg/l, while lethal effects in fish occur in DO concentrations from 0.5 to 3.0 mg/l (Vaquer-Sunyer and Duarte 2008). For example, in the Chesapeake Estuary in the mid-Atlantic region of the east coast of the United States, demersal fish abundance and diversity (richness) greatly decreased at DO concentrations ≤ 4 mg/l (Buchheister et al. 2013). However, some fish species may remain in affected areas due to differences in physiological tolerance to low DO conditions (Pihl et al. 1991; Breitburg et al. 1994; Bell and Eggleston 2005) and due to relatively limited mobility that may limit their ability to escape low DO areas (Lewis et al. 2021).

Changes in the spatial distribution of fish species can result in an overlap in predators and prey, thereby influencing the quality and extent of nursery habitats (Hanks and Secor 2011). As such, determining spatial changes in fish distribution and abundance can aid in identifying and evaluating spatially-based management approaches and monitoring strategies, as well as defining where anthropogenic activities have an impact in coastal ecosystems. Ultimately, a better understanding of fish dynamics at various spatial scales is required (Craig and Bosman 2013).

In the warm temperate region of South Africa, the Swartkops River flows through a highly urbanised and industrialised region of the Eastern Cape (Adams et al. 2019; Scharler and Baird 2005), with untreated municipal wastewater entering the estuary through diffuse storm water runoff from informal settlement areas (Adams et al. 2020). As such, the water that flows into the Swartkops Estuary contains a variety of pollutants and inorganic dissolved nutrients, which contribute to high dissolved inorganic nitrogen and phosphate concentrations in the estuary (Scharler and Baird 2003; Scharler and Baird 2005). Because of reduced vertical mixing and strong stratification, persistent eutrophic conditions dominate, with frequent phytoplankton blooms (> 20 μg Chl *a* l^{-1}) occurring from the middle reaches to the tidal head of the estuary where hypoxic conditions also occur (Adams et al. 2019).

The Sundays Estuary is surrounded by agricultural activities and has been identified as a permanently eutrophic system. Owing to the substantial citrus farming in its catchment area, this estuary has highly modified freshwater inflow patterns and nutrient-rich irrigation return flows (Lemley et al. 2017; Lemley et al. 2018). Water temperature profiles from surface to bottom vary strongly with season, with maximum temperatures recorded in summer. Dissolved oxygen concentrations are usually lower in summer than other seasons with hypoxic conditions (< 2 mg/l) frequently occurring in the middle reaches (Lemley et al. 2017) in the Sundays Estuary. Furthermore, red-tide events containing the harmful algal bloom species *Heterocapsa rotundata* (Heterocapsaceae) and *Heterosigma akashiwo* (Chattonellaceae) have been observed in this system earlier by Hilmer and Bate (1990) and recently by Lemley et al. (2017) during winter, spring and summer. Lemley et al. (2017) mentioned that the frequent occurrence of these two species (> 100 μg Chl *a* l^{-1}) indicate an increasing magnitude of their existence in this system (Lemley et al. 2017). During the decay phase of these HABs, DO shifts from supersaturated levels (> 10 mg/l) during peak HABs to occurrences of bottom low DO (2–4 mg/l), notably in the middle reaches of the estuary (Lemley et al. 2018). Intense HABs result in the occurrence of hypoxia (DO < 2 mg/l) (Hallett et al. 2016).

Although both estuaries serve as important nursery habitats for several demersal fish species (see Chapter Five), there is limited available information on how fish communities respond to low DO events (mostly associated with HABs) in the Sundays and Swartkops estuaries. A recent study (Smit et al. 2020) conducted in the Sundays Estuary provides insight into the fine-scale consequences that HABs have on the larvae of *Gilchristella aestuaria* (Clupeidae), a highly mobile pelagic species. They found that during HABs, *G. aestuaria* were recorded at low densities and mainly consisted of late larval stages. With increasing anthropogenic pressures (characteristic of the Anthropocene epoch) placed on estuarine fish communities, knowledge of species-specific responses to low dissolved oxygen is important for the development of effective estuarine management plans.

Bi-seasonal sampling in the Swartkops and Sundays estuaries allowed for the quantification of low dissolved oxygen and hypoxic events in both estuaries during the study period. The aim of this chapter was to (i) characterize low DO and hypoxic events in both estuaries and (ii) determine the impact of low DO and hypoxic events on overall fish species abundance and richness as well as spatial shifts in the distribution of selected demersal species in these heavily

polluted estuaries during these events. Based on existing evidence in the literature and rationale presented in the introduction, it was hypothesized that:

(i) Fish abundance and species richness will decrease due to low DO and hypoxic events.

(ii) CPUE of each species will decrease in low DO areas.

(iii) Hypoxic (< 2 mg/l) zones (mostly accompanied by HABs) will be avoided by demersal species, with species aggregation occurring on the edge of these zones.

6.2 Methods and Materials

6.2.1 Sampling

Demersal fishes were sampled in the Sundays and Swartkops estuaries between February 2018 and September 2019 for a total of eleven sampling occasions. Study site and sampling methods are described in detail in Chapter Two and Three. Dissolved oxygen (mg/l) were recorded at each sampling site using a YSI (6290) multi-parameter probe. Both surface and bottom waters were measured but, for analysis, in this chapter, only bottom values were used.

As in Chapter Four catch-per-unit-effort (CPUE) was calculated as the index of abundance (numbers per 1000 m^2):

$$CPUE = total\ number\ of\ individuals\ at\ each\ site \div area\ trawled \times 1000$$

6.2.2 Data analysis

To assess differences in DO among sampling sites for each month, the data were tested for normality using a normal probability plot and Shapiro-Wilk test and homogeneity of variance was tested using a Levene's test. Since the assumptions of normality were not met, a non-parametric Kruskal-Wallis (H) ANOVA was used for this analysis. These analyses were conducted in the R environment for computing statistics version 3.6.0 (R Development Core Team 2019). To spatially visualise differences in DO among sites, mean DO throughout the study period in each site in both estuaries was plotted in ArcGIS (Arc GIS v. 10.3.1).

Low DO events that occurred during the sampling period (between February 2018 and September 2019) in the two estuaries were identified. The classification and criteria used to identify these events were obtained from the existing literature (e.g. Craig and Bosman 2013). When DO concentrations declined below 4 mg/l at any site (then that particular site was classified as having a low DO event during the sampling peroid), the sampling occasion was classified as a low DO event and when DO levels decreased below 2 mg/l then it was classified as a hypoxic event (then that particular site was classified as hypoxic).

To evaluate differences in DO among sites in the Swartkops and Sundays etuaries, ANOVA was used. Diagnostics plots on the model residuals were run to assess the ANOVA assumptions, followed by the Shapiro-Wilk test of normality and Levene's test of homogeneity of variance. The assumptions of normality and homogeneity of variance were violated; DO was $\log_{10}(x+1)$ transformed. Post-hoc pairwise multiple comparisons were performed using Tukey's HSD test when there were significant differences ($P < 0.05$) among sites to determine which means were significantly different from one another. These analyses were conducted using the '*mass*' package in the R environment for computing statistics version 3.6.0 (R Development Core Team 2019).

To assess differences in CPUE among sampling months/occasions and sites in both estuaries, a non-parametric Kruskal-Wallis (H) ANOVA was used as the CPUE data (numbers per 1000 m^2) failed the assumptions of normality (using a normal probability plot and Shapiro-Wilk test) and homogeneity of variance (using a Levene's test). Post-hoc pairwise multiple comparisons were performed using Dunn's test when there were significant differences ($P < 0.05$) among sampling months. The total number of species in each sampling month and at each site was also calculated in the Sundays and Swartkops estuaries. These analyses were conducted in the R environment for computing statistics version 3.6.0 (R Development Core Team 2019).

To determine which species could potentially be affected by low DO events, the overall dominant species in terms of CPUE recorded in both estuaries during the study period were selected. These included *Galeichthys feliceps* (3.3 fish per 1000 m^2), *Pomadasys olivaceus* (1.4 fish per 1000 m^2), *Rhabdosargus holubi* (67.3 fish per 1000 m^2), *Solea turbynei* (50.5 fish per 1000 m^2), *Heteromycteris capensis* (28.7 fish per 1000 m^2), *Caffrogobius gilchristi* (54.3 fish per 1000 m^2), *Psammogobius knysnaensis* (45.5 fish per 1000 m^2) and *Glossogobius callidus* (43.2 fish per 1000 m^2). The number of individuals of each species recorded at each site during the study period in each sampling occasion were visually assessed using boxplots plotted in the R environment for computing statistics version 3.6.0 (R Development Core Team 2019).

In order to assess species-specific avoidance of low DO, the distribution and abundance (mean CPUE) (CPUE recorded throughout the study period and averaged per site) of the selected species during low DO and hypoxic events were visually assessed using ArcMap (Arc GIS v. 10.3.1). Spatial analyses were carried out using geo-processing tools in ArcMap (Arc GIS v. 10.3.1). For this analysis, spatial interpolation analysis Inverse Distance Weighting (IDW) was

used to interpolate the overall mean DO (average per site) and low DO events/months identified in each estuary. The IDW is more suitable for interpolation of physical data in coastal areas than Kringing (Radiarta et al. 2006). The DO values in each site were interpolated to the second power.

6.3 Results

6.3.1 Spatio-temporal variability in dissolved oxygen

In the Sundays Estuary, DO concentrations were consistently lower in the middle reaches (specifically Site S5 & S6) than the lower and upper reaches (Figure 6.1a). There were significant diffrences among sites (ANOVA: $F_{(8, 90)} = 3.3$; $P = 0.002$), with S6 significantly different from S1 and S2 (Tukey's HSD test; $P > 0.05$). The lowest mean DO (4.7 mg/l) was recorded at Site S6 and the highest mean DO (8.0 mg/l) was observed at Site S1 (Figure 6.1a). The lowest (0.5 mg/l - hypoxic) and highest (10 mg/l – supersaturated) DO concentrations were measured at S5 (Figure 6.1a). In the Swartkops Estuary, DO concentrations decreased from the mouth to the upper reaches of the estuary, with the lowest mean DO (4.2 mg/l) recorded at Site SW7 (uppermost site) and the highest mean DO (7.6 mg/l) measured at site SW1 (mouth) (Figure 6.1b). The lowest DO (2.5 mg/l) was recorded at SW7 (uppermost site) with the highest (8.3 mg/l) at SW2 (Figure 6.1b). Significant differences among sites (ANOVA: $F_{(6, 70)} = 11.2$; $P = 0.001$) were observed, with SW1 significantly different from SW5, SW6 and SW7 (Tukey's HSD test; $P > 0.05$).

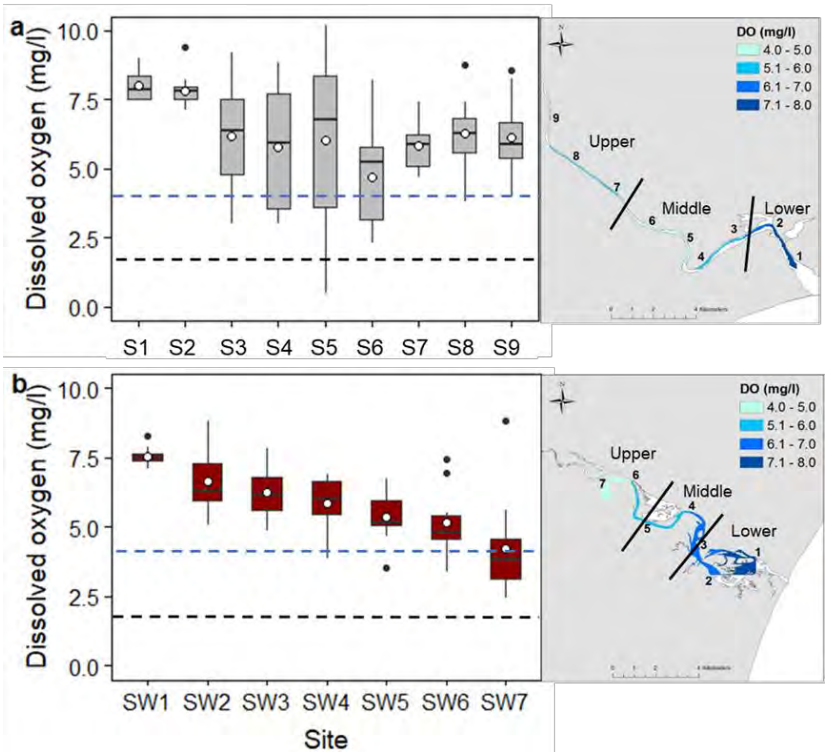

Figure 6.1: Box plots showing median (thick solid lines) and mean (white circles) bottom water dissolved oxygen (DO) (mg/l) at each site in (a) the Sundays Estuary and (b) the Swartkops Estuary during the sampling period (February 2018 – September 2019). Boxes represent the upper and lower limits of the third and first quartiles and whiskers represent the range. Black dots represent outliers. Blue dotted line represents low DO (4 mg/l) and the black dotted line represents hypoxic (2 mg/l) conditions.

In the Sundays Estuary, low DO concentrations (< 4 mg/l) were recorded in February 2018, March 2018, November 2018, February 2019 and April 2019. The lowest DO (0.5 mg/l) indicative of hypoxia was measured in February 2019 (Figure 6.2a). In the Swartkops Estuary, low DO events (< 4 mg/l) were measured in February 2018, March 2018, May 2018, February 2019 and September 2019, with the lowest DO (2.6 mg/l) measured in October 2018 (Figure 6.2b). Four low DO events were selected in the Sundays (February 2018, March 2018, April 2019 and February 2019) and Swartkops (February 2018, March 2018, May 2018 and October 2018) estuaries for further analysis.

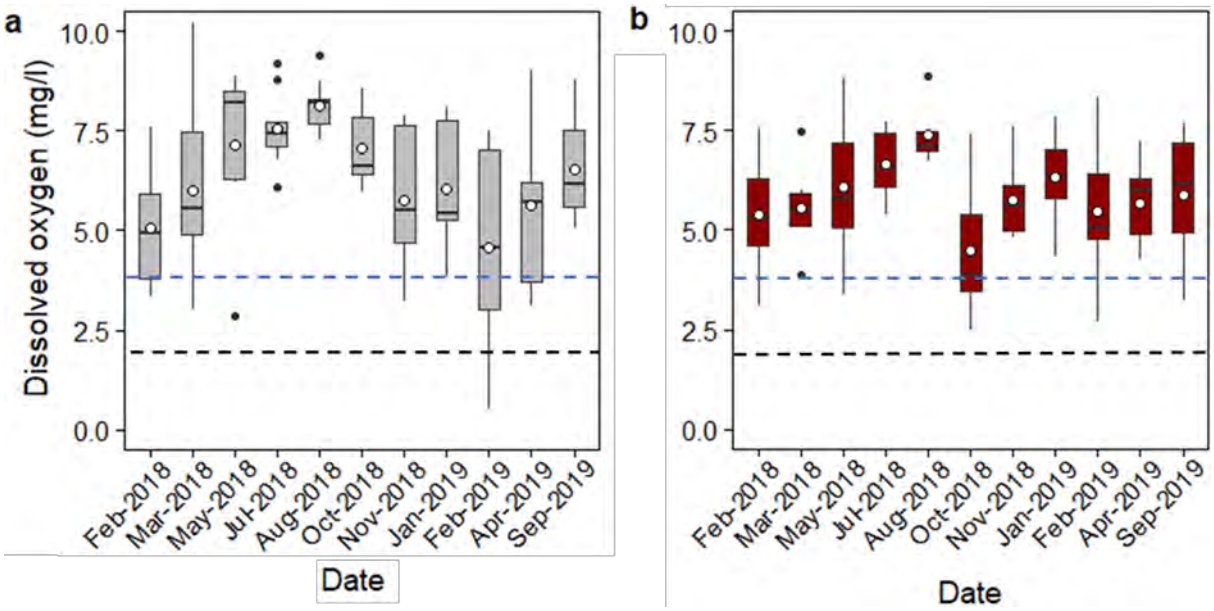

Figure 6.2: Box plots showing median (thick solid lines) and mean (white circles) monthly bottom water dissolved oxygen (DO) in the (a) Sundays and (b) Swartkops estuaries during the sampling period (February 2018 – September 2019). Boxes represent the upper and lower limits of the third and first quartiles, and whiskers represent the range. Black dots represent outliers. Blue dotted line represents low DO (4 mg/l) and the black dotted line represents hypoxic (2 mg/l) conditions.

6.3.2 Effect of low DO on species richness and abundance

In the Sundays Estuary, the overall mean CPUE of fish was lowest during the winter months of July 2018 (100 fish per 1000 m^2) and August 2018 (78 fish per 1000 m^2) and higher during the warmer months of October 2018 (322 fish per 1000 m^2) and January 2019 (418 fish per 1000 m^2). A decline in mean CPUE recorded during the summer low oxygen event in February

2019 (125 fish per 1000 m^2) was also observed. There were significant differences among sampling months ($H = 23.3$: df = 10, $P = 0.001$), with July 2018 and August 2018 significantly different from October 2018 and January 2019 (Post hoc test; $P < 0.05$) (Figure 6.3a). The number of species recorded was lowest in the low DO months of February 2018 (12 species), March 2018 (11 species) and April 2019 (11 species) (Figure 6.3a). The number of species recorded was also low in July 2018 (10 species) and highest in the summer month of January 2019 (17 species) (Figure 6.3a).

In the Swartkops Estuary, mean CPUE of fish (average per month) was highest in November 2018 (354 fish per 1000 m^2) and February 2019 (325.7 fish per 1000 m^2) and lowest (< 113 fish per 1000 m^2) during the low DO events in February 2018, March 2018, May 2018, and October 2018 (Figure 6.3). Low CPUE was also observed in August 2018 (Figure 6.3b). Significant differences in CPUE among sampling months ($H = 18.87$: df = 10, $P = 0.04$) were observed, with low DO sampling months (February 2018, March 2018, May 2018, and October 2018) significantly lower than November 2018 and February 2019 (Post hoc test; $P < 0.05$) (Figure 6.3b). Species richness also varied among sampling months, with the highest species richness recorded in November 2018 (13 species) and January 2019 (11 species) and the lowest recorded in July 2018 and October 2018 (seven species). The October 2018 low species richness coincided with the lowest DO event (Figure 6.3b).

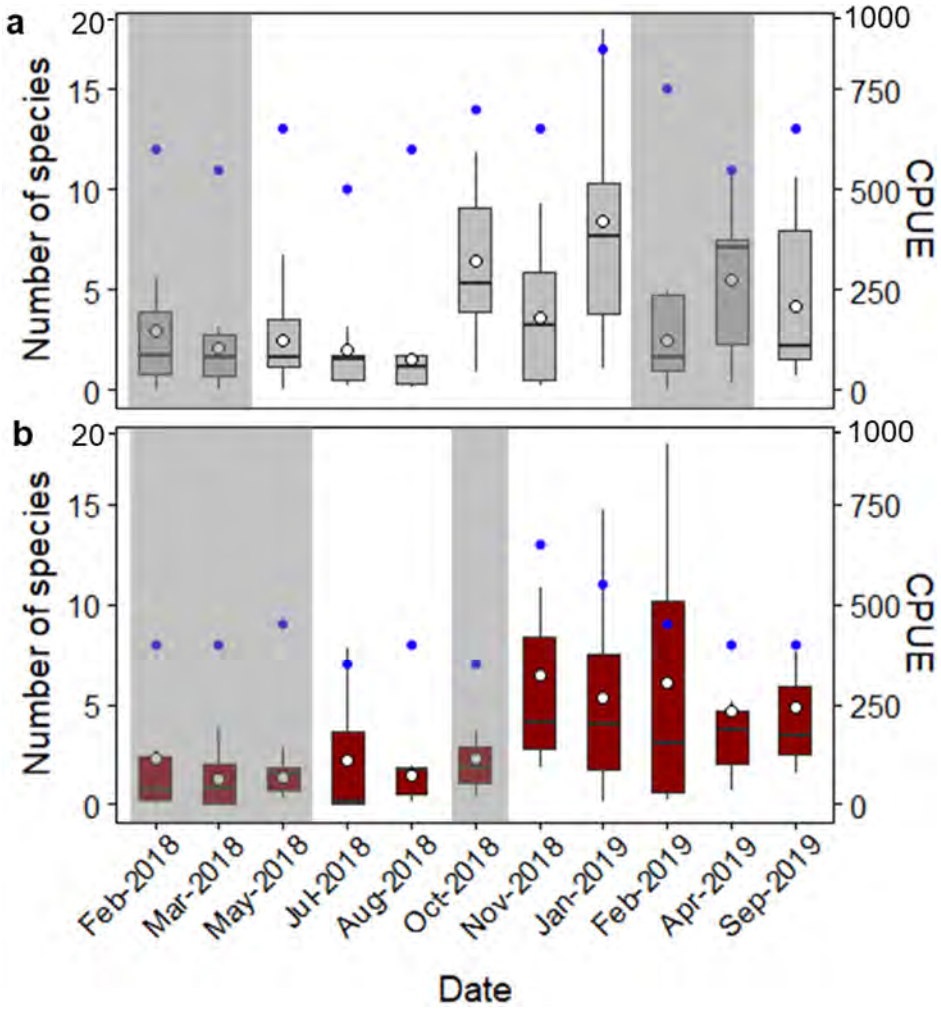

Figure 6.3: The number of species (blue circles) and catch-per-unit-effort (CPUE) (fish per 1000 m^2) (box plots) recorded monthly in the (a) Sundays Estuary and (b) Swartkops Estuary during the sampling period (February 2018 – September 2019). Boxes represent the upper and lower limits of the third and first quartiles, and whiskers represent the range; and thick solid lines the median. White circles represent the mean. Grey shading indicates low DO events.

In terms of sampling sites, the overall CPUE of fish in the Sundays Estuary was significantly lower in sites affected by low DO than other sites ($H = 27.7$, df = 8, $P = 0.001$) (Figure 6.4a). The mean CPUE was consistently lower at S5 (100 fish per 1000 m^2), S6 (180 fish per 1000 m^2), S7 (84 fish per 1000 m^2) as well as S8 (89 fish per 1000 m^2) and significantly higher at S1 (296 fish per 1000 m^2), S2 (229 fish per 1000 m^2) and S3 (355 fish per 1000 m^2) (Figure 6.4a). The number of species recorded was also lowest in the low DO sites, namely S5 (nine species), S6 (nine species) and S7 (eight species) and highest at S3 (17 species) (Figure 6.4a).

In the Swartkops Estuary, significant differences in CPUE were observed among sites (H = 16.07, df = 6, P = 0.01), with CPUE recorded at SW7 significantly higher than SW1 and SW3 ($P < 0.005$). Mean CPUE was higher at SW7 (388 fish per 1000 m^2) than other sites and lower at SW1 (62 fish per 1000 m^2) and SW3 (66 fish per 1000 m^2) (Figure 6.4b). The number of species was highest at SW4 (14 species) and SW5 (13 species) and lowest at SW1 (nine species) and SW2 (nine species) (Figure 6.4b). A decrease in species richness in the low DO site (SW7) (10 and 11 species) was also observed (Figure 6.4b).

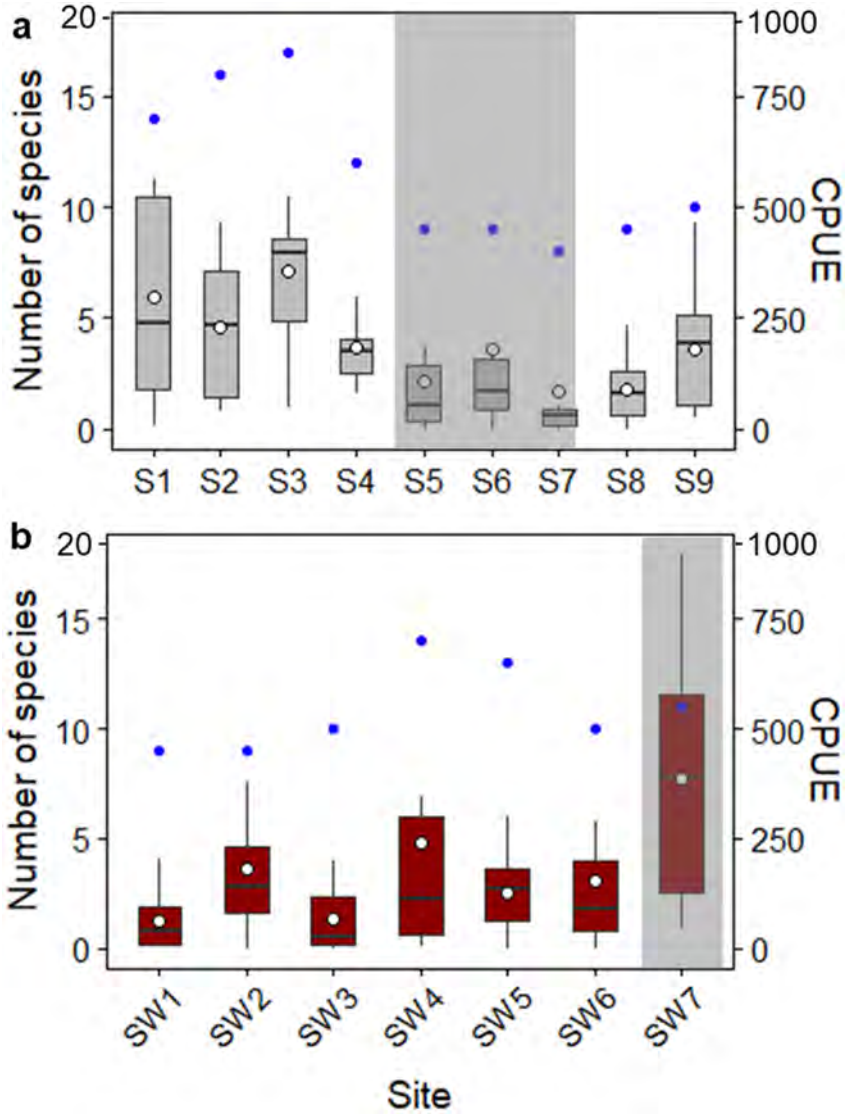

Figure 6.4: The number of species (blue circles) and catch-per-unit-effort (CPUE) (fish per 1000 m^2) (box plots) recorded monthly in the (a) Sundays Estuary and (b) Swartkops Estuary during the sampling period (February 2018 – September 2019). Boxes represent the upper and lower limits of the third and first quartiles, and whiskers represent the range; and thick solid lines the median. White circles represent the mean. Grey shading indicates low DO events.

6.3.3 Dominant marine estuarine-opportunist, marine estuarine-dependent and estuarine (E&M and E&F) species

In the Sundays Estuary, among the species dominant in terms of CPUE, *Pomadasys commersonnii* (MED), *Rhabdosargus holubi* (MED) and *Solea turbynei* (MED) were consistently abundant in the low DO middle reaches of the estuary during the sampling period. *Galeichthys feliceps* (MEO), *Heteromycteris capensis* (MED), *Caffrogobius gilchristi* (E&M), and *Psammogobius knysnaensis* (MEO) occurred in higher numbers in the lower reaches of the estuary and *Glossogobius callidus* (E&F) was recorded in the uppermost sites (Figure 6.5). In the Swartkops Estuary, although relatively few individual *P. commersonnii* were recorded during the study period, they occurred mostly in the upper reaches. *Psammogobius knysnaensis* was more abundant in the lower reaches of the estuary. *Rhabdosargus holubi, S. turbynei, C. gilchristi* and *G. callidus* occurred mostly in the upper reaches where low DO events were recorded (Figure 6.6).

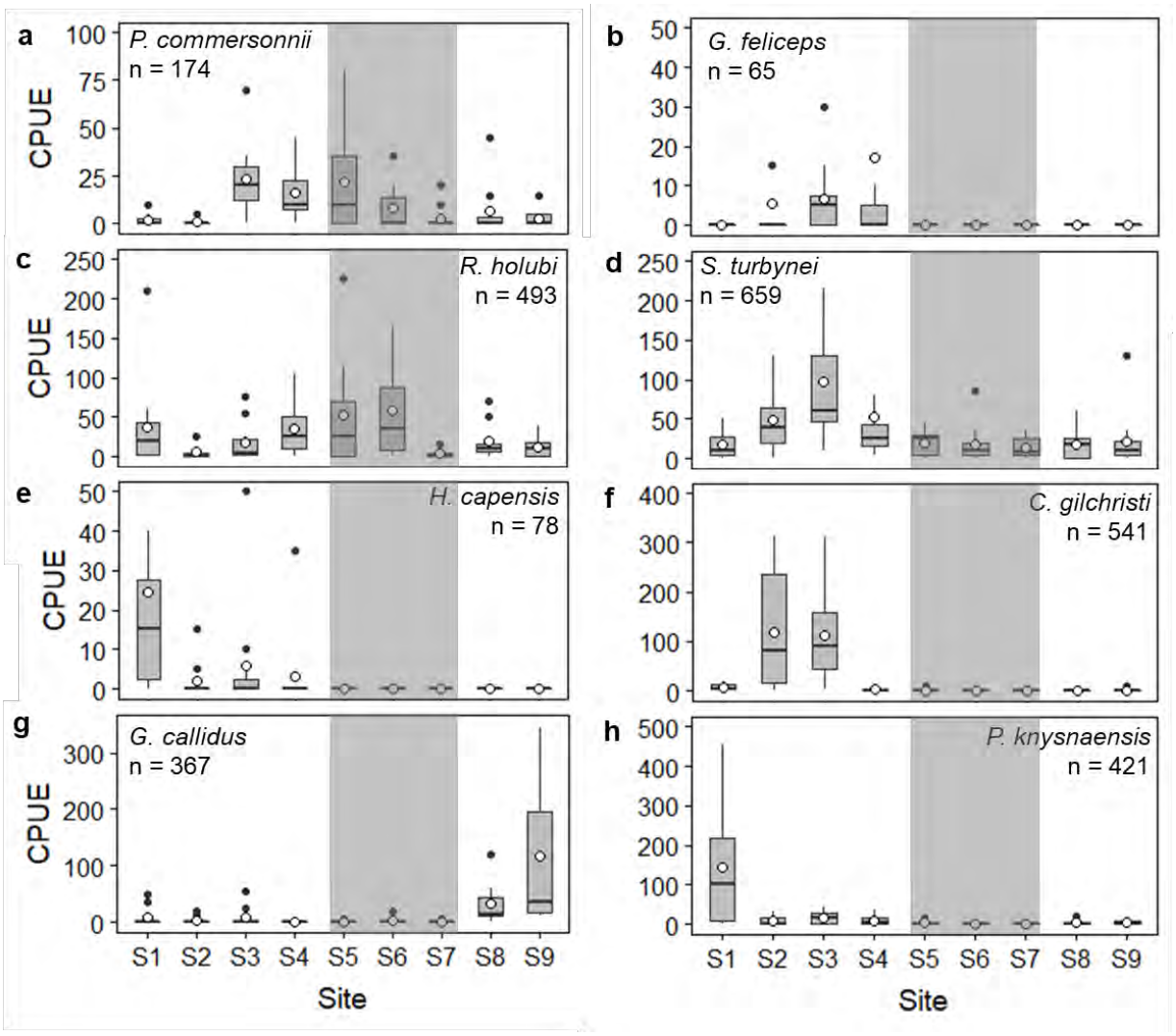

Figure 6.5: Catch-per-unit-effort (CPUE) (fish per 1000 m^2) of (a) *Pomadasys commersonnii* (b) *Galeichthys feliceps* (c) *Rhabdosargus holubi,* (c) *Solea turbynei,* (e) *Heteromycteris capensis* (f) *Caffrogobius gilchristi* (g) *Glossogobius callidus* and (h) *Psammogobius knysnaensis* in the Sundays Estuary during the sampling period (February 2018 – September 2019). Boxes represent the upper and lower limits of the third and first quartiles, and whiskers represent the range; and thick solid lines the median. Black dots represent outliers and white circles represent the mean. Grey shading indicates low DO events.

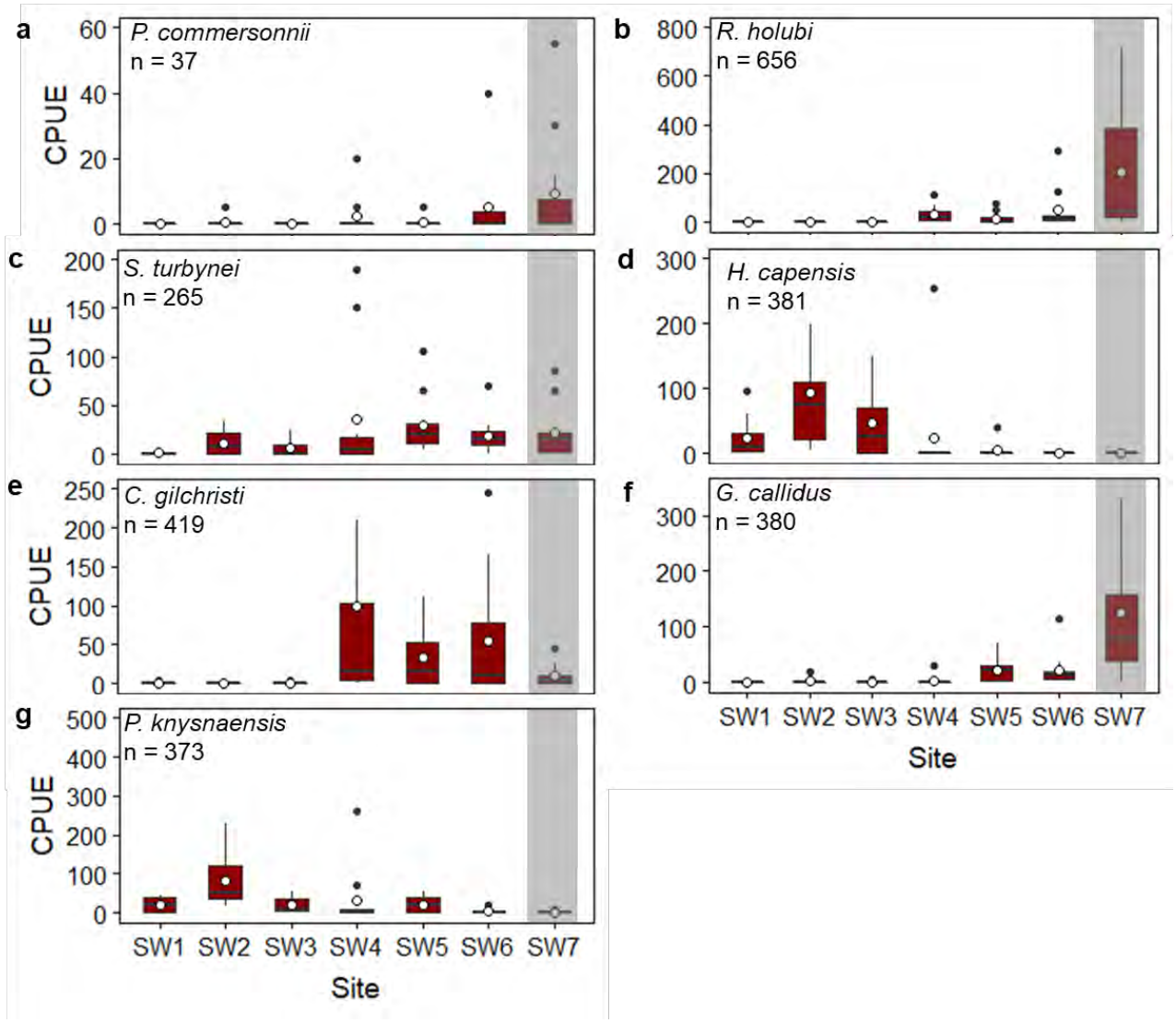

Figure 6.6: Catch-per-unit-effort (CPUE) (fish per 1000 m^2) of (a) *Pomadasys commersonnii* (b) *Rhabdosargus holubi,* (c) *Solea turbynei,* (d) *Heteromycteris capensis* (e) *Caffrogobius gilchristi* (f) *Glossogobius callidus* and (g) *Psammogobius knysnaensis* in the Swartkops Estuary during the sampling period (February 2018 – September 2019). Boxes represent the upper and lower limits of the third and first quartiles, and whiskers represent the range; and thick solid lines the median. Black dots represent outliers and white circles represent the mean. Grey shading indicates low DO events.

6.3.4 Effect of low DO on dominant marine estuarine-opportunist, marine estuarine-dependent and estuarine (E&M and E&F) species.

Species that consistently occurred (species that were recorded in each sampling trip and utilised most sites of the estuary) in the low DO middle reaches of the Sundays Estuary and the low DO upper reaches of the Swartkops Estuary (Figure 6.5 & 6.6) were selected for further spatial analyses. Based on this criteria, three species were identified in the Sundays Estuary and four in the Swartkops Estuary. In the Sundays Estuary, *R. holubi*, *S. turbynei*, and *P. commersonnii* (mostly recorded in the Sundays Estuary) were selected. In the Swartkops Estuary, *R. holubi, S. turbynei, C. gilchristi* and *G. callidus* were selected.

The selected species were all affected by low DO events in both estuaries, and particularly during the hypoxic conditions which only occurred in the Sundays Estuary. Species were either absent from the affected sites or decreased in abundance (CPUE), with species aggregation occurring on the edge of the low DO areas/sites. The overall mean CPUE (recorded throughout the study period and averaged per site) for each dominant species was compared with mean CPUE recorded in each site during low DO and hypoxic events/months.

In the Sundays Estuary, a decrease in mean CPUE for *P. commersonnii* was observed in low DO (< 4 mg/l) sites in April 2019, with zero catches at DO levels of 0.5 mg/l (hypoxia) in February 2019 and 9 – 10 mg/l (supersaturation indicative of HABs) in March 2018 in the middle reaches of the estuary (Figure 6.7). *Rhabdosargus holubi* had lower CPUE in Site S5, which had hypoxic conditions (0.5 mg/l) in February 2019 and the supersaturation indicative of a HAB (9 – 10 mg/l) in March 2018 in the middle reaches, with individuals recorded in the adjacent sites at DO concentrations above 1.1 mg/l (Figure 6.8). Higher catches (mean CPUE) of both *P. commersonnii* and *R. holubi* were observed in the low DO sites in February 2018 (Figure 6.7 & 6.8). Although, there was a decrease in CPUE for *Solea turbynei* in low DO sites (< 4 mg/l) in February 2018, individuals were distributed throughout the estuary in April 2019 even in low DO sites (< 4 mg/l). Zero catches were recorded at sites with supersaturated DO (9 – 10 mg/l) in March 2018 and hypoxic conditions (0.5 mg/l) in February 2019 (Figure 6.9).

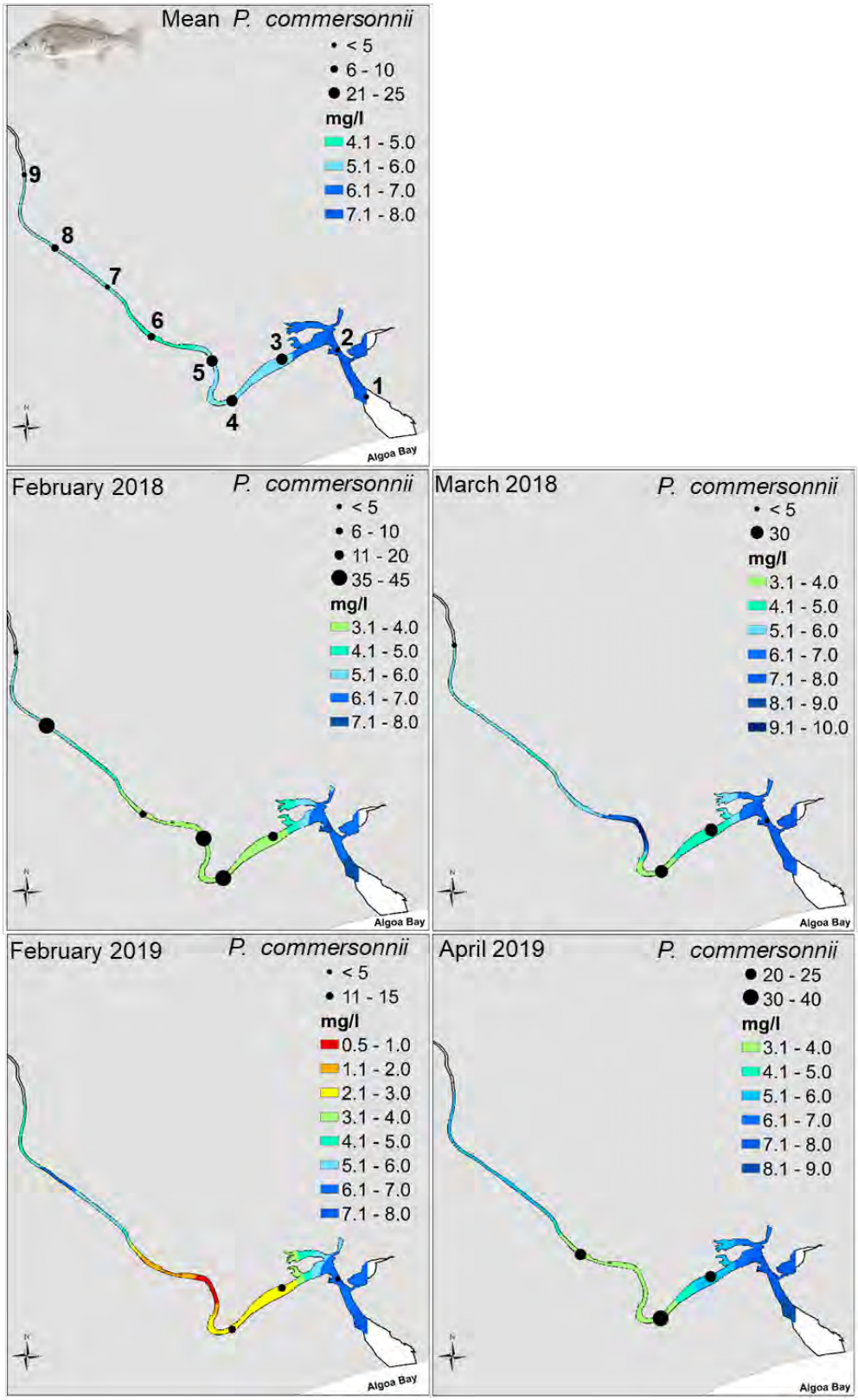

Figure 6.7: The mean catch-per-unit-effort (CPUE) (fish per 1000 m^2) recorded during the study period and CPUE recorded during low DO events that occurred in February 2018, March 2018, February 2019 and April 2019 for *Pomadasys commersonnii* in the Sundays Estuary during the sampling period (February 2018 – September 2019).

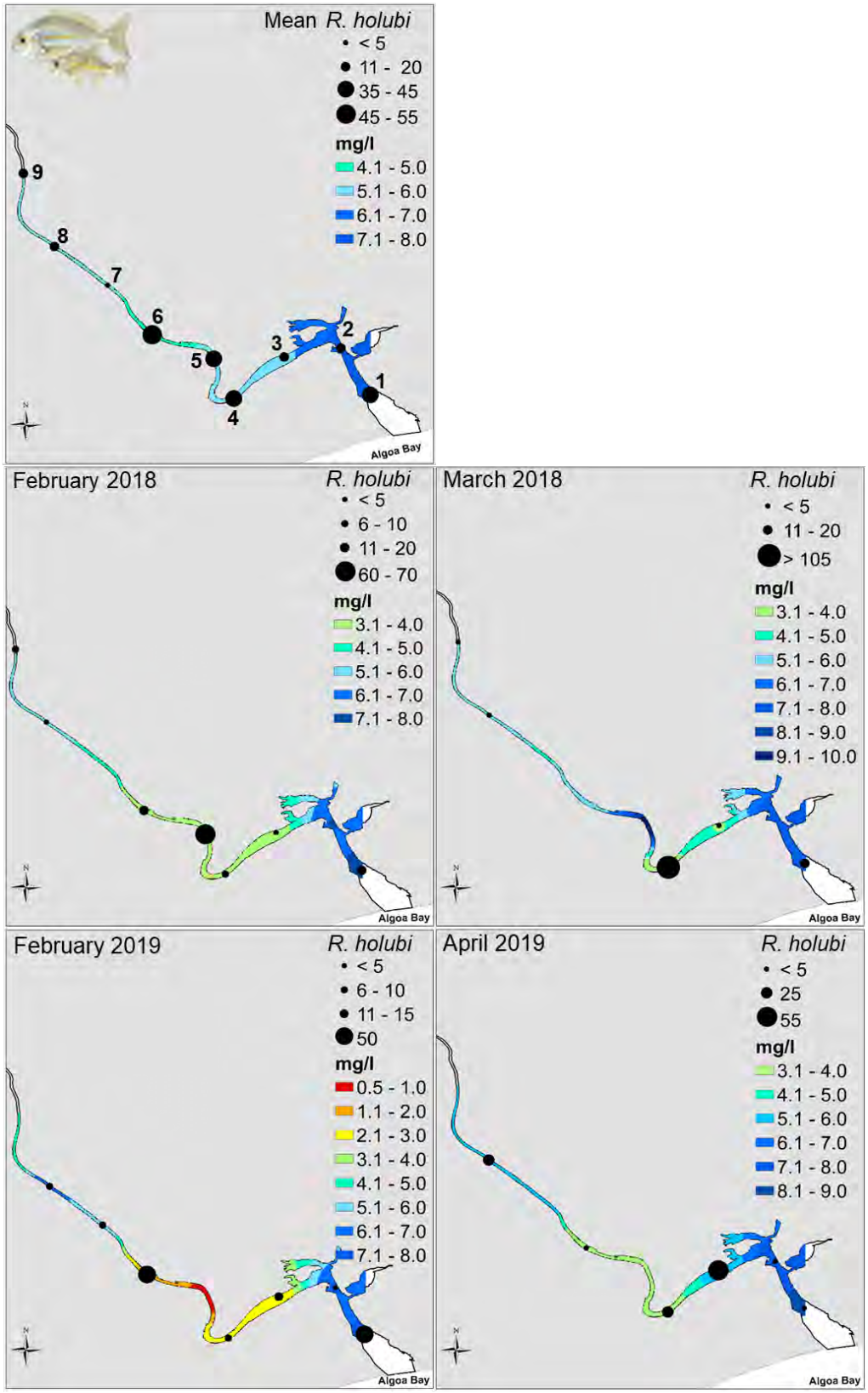

Figure 6.8: The mean catch-per-unit-effort (CPUE) (fish per 1000 m^2) recorded during the study period and CPUE recorded during low DO events that occurred in February 2018, March 2018, February 2019 and April 2019 for *Rhabdosargus holubi* the Sundays Estuary during the sampling period (February 2018 – September 2019).

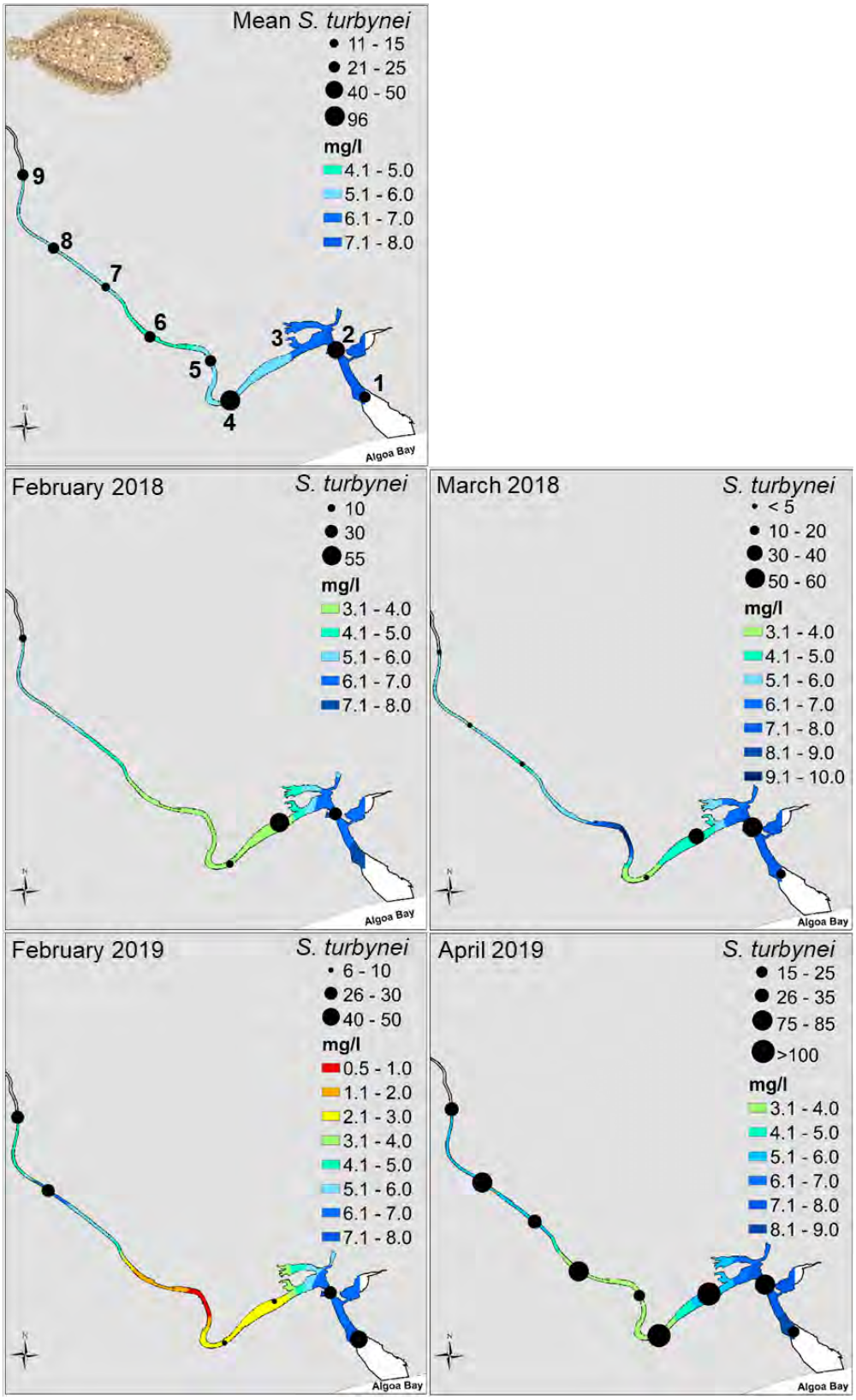

Figure 6.9: The mean catch-per-unit-effort (CPUE) (fish per 1000 m^2) recorded during the study period and CPUE recorded during low DO events that occurred in February 2018, March 2018, February 2019 and April 2019 for *Solea turbynei* in Sundays Estuary during the sampling period (February 2018 – September 2019).

In the Swartkops Estuary, although CPUE for all the selected species decreased in sites where low DO (< 4 mg/l) was recorded, lower CPUE were still recorded in these sites. *Rhabdosargus holubi* decreased in abundance in the affected sites in February 2018, May 2018 and October 2018, while in March 2018 the CPUE remained high in these sites (Figure 6.10). The CPUE for *S. turbynei* declined in March 2018, May 2018 and October 2018 with individuals only avoiding low DO sites in February 2018, when DO was < 3 mg/l (Figure 6.11). *Caffrogobius gilchristi* was also affected by low DO, with CPUE decreasing in the upper reaches particularly in March 2018, May 2018 and October 2018 (Figure 6.12). *Glossogobius callidus* decreased in abundance at DO concentrations ranging between 3 and 4 mg/l recorded in March 2018 and May 2018, while in February 2018 and October 2018 CPUE remained high in low DO sites (Figure 6.13).

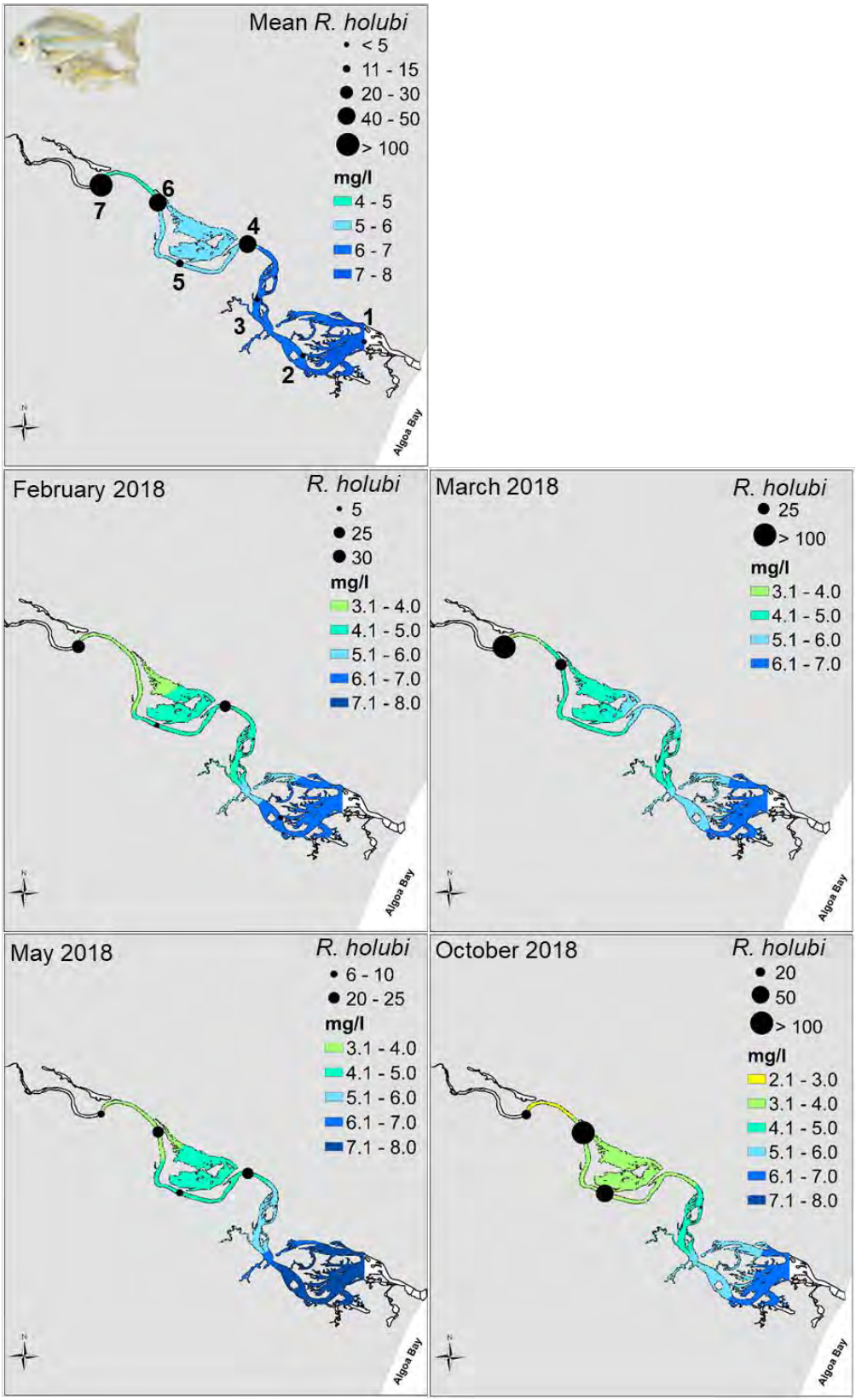

Figure 6.10: The mean catch-per-unit-effort (CPUE) (fish per 1000 m^2) recorded during the study period and CPUE recorded during low DO events that occurred in February 2018, March 2018, May 2018 and October 2018 for *Rhabdosargus holubi* in the Swartkops Estuary during the sampling period (February 2018 – September 2019).

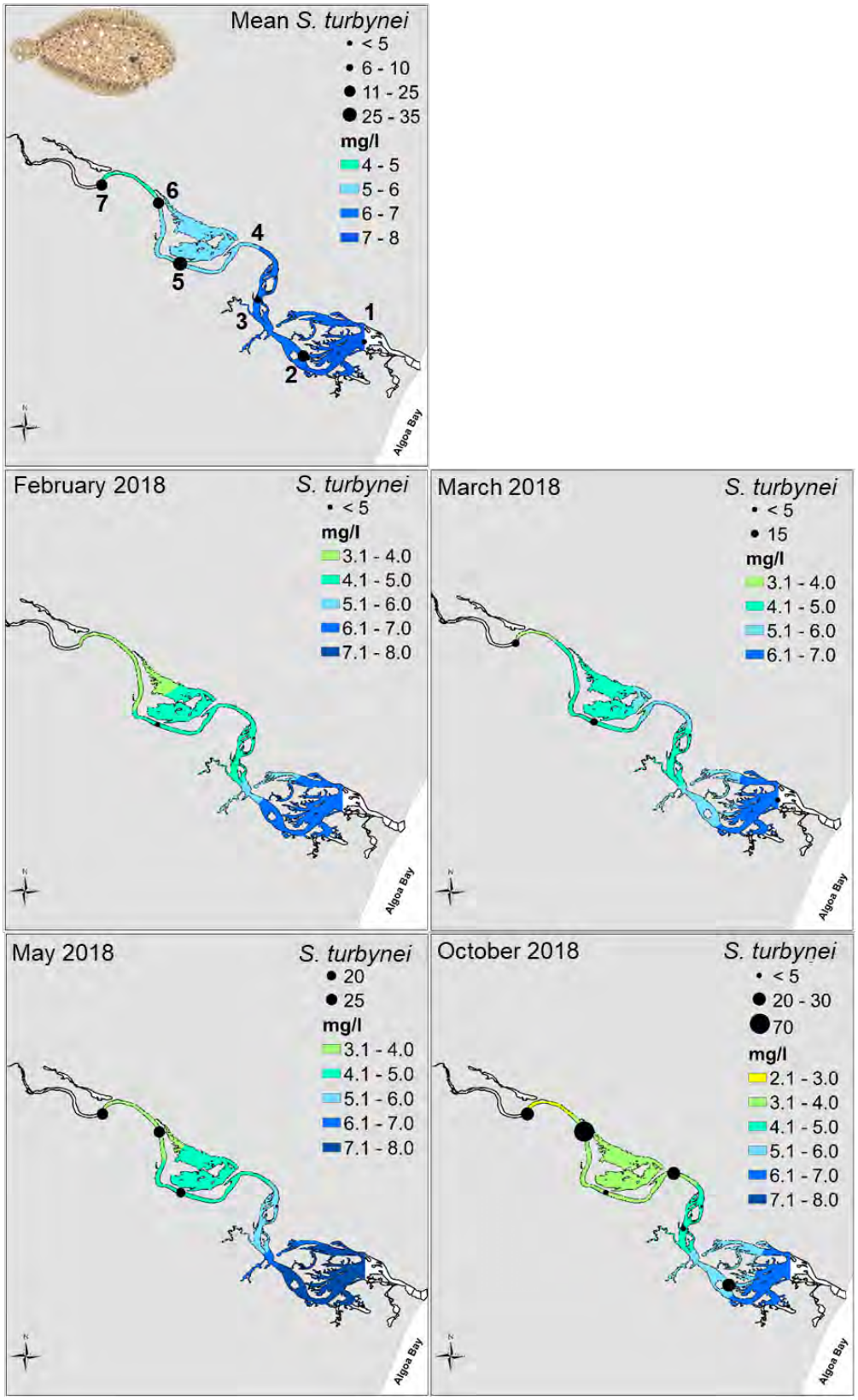

Figure 6.11: The mean catch-per-unit-effort (CPUE) (fish per 1000 m^2) recorded during the study period and CPUE recorded during low DO events that occurred in February 2018, March 2018, May 2018 and October 2018 for *Solea turbynei* in the Swartkops Estuary during the sampling period (February 2018 – September 2019).

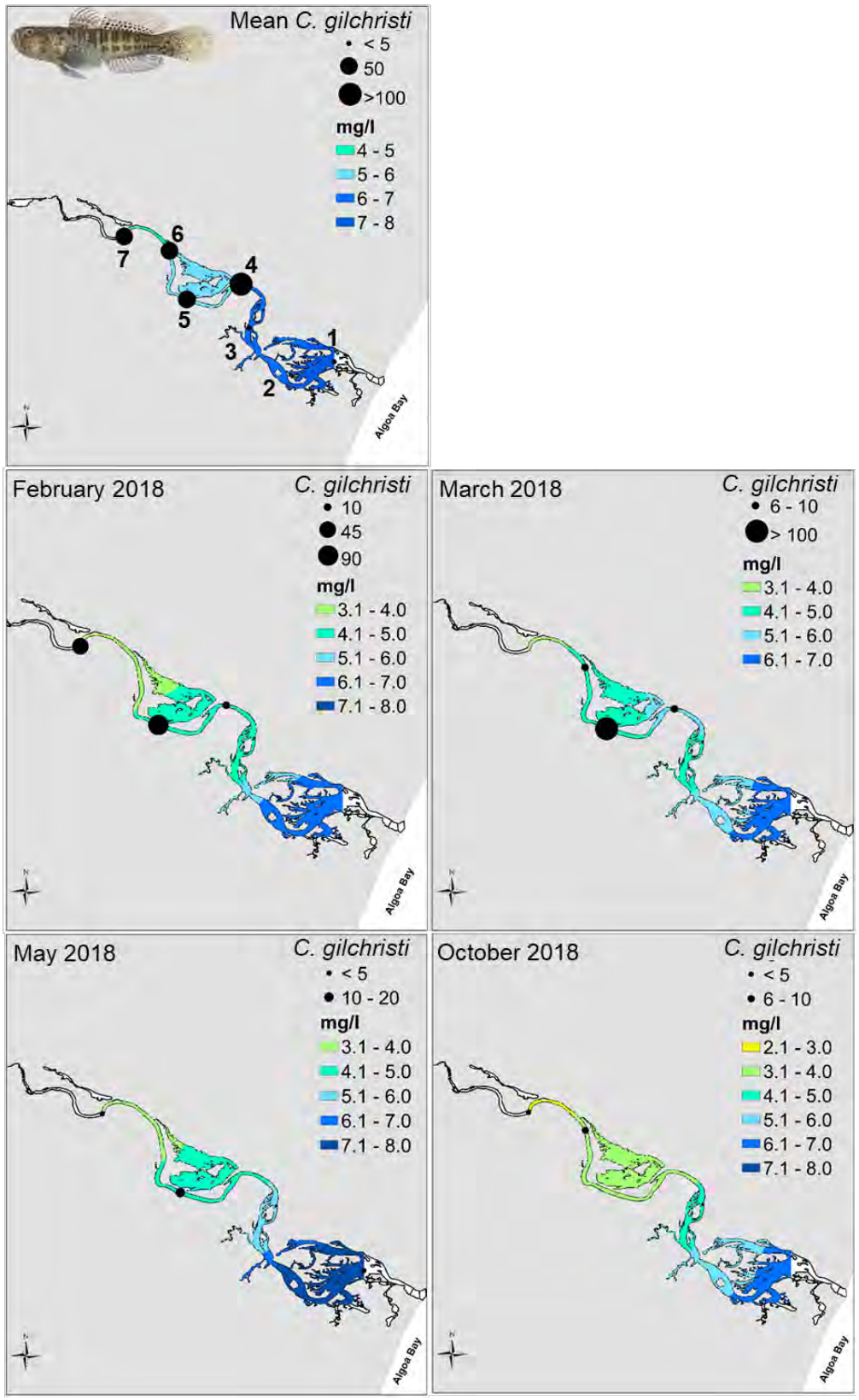

Figure 6.12: The mean catch-per-unit-effort (CPUE) (fish per 1000 m^2) recorded during the study period and CPUE recorded during low DO events that occurred in February 2018, March 2018, May 2018 and October 2018 for *Caffrogobius gilchristi* in the Swartkops Estuary during the sampling period (February 2018 – September 2019).

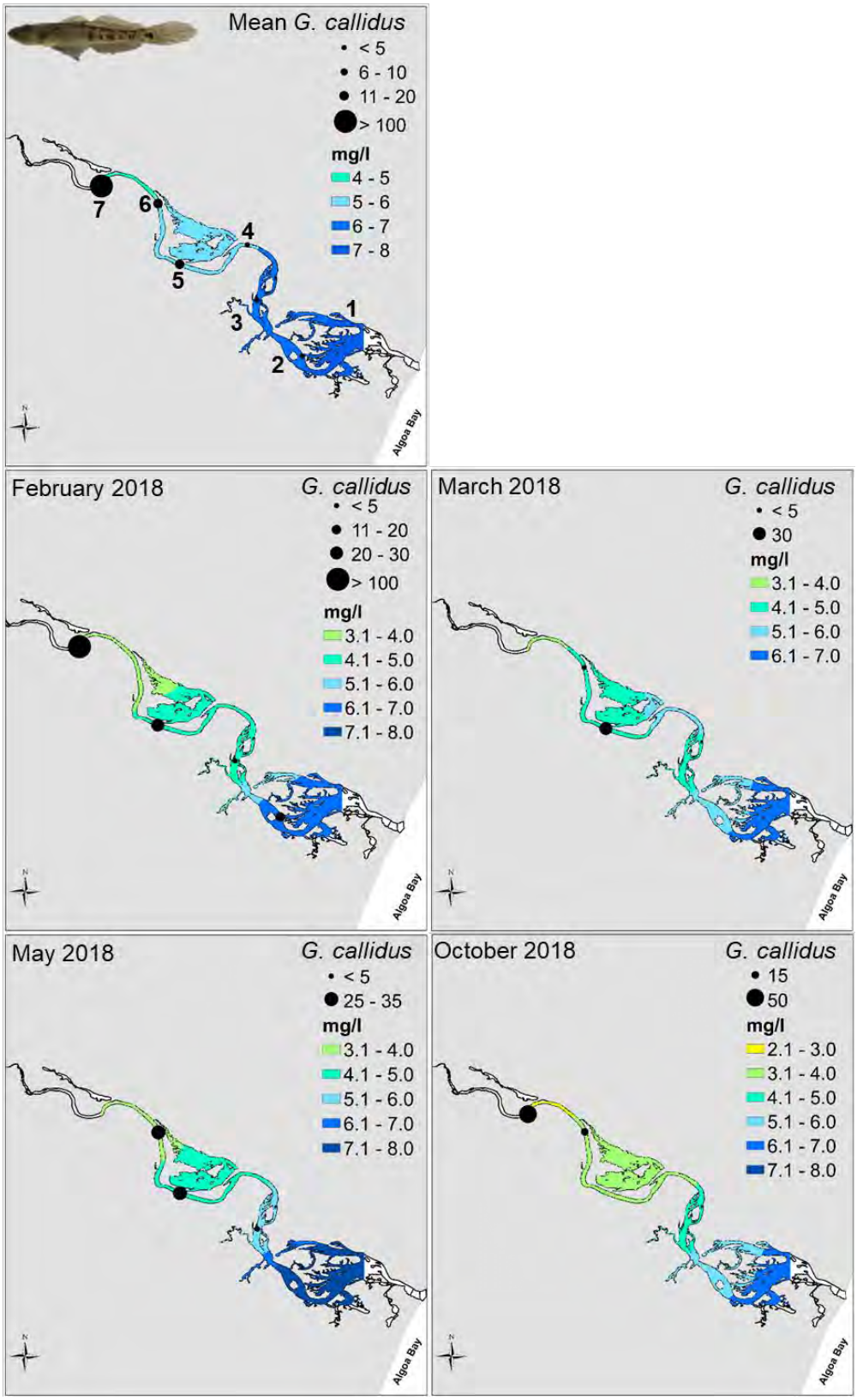

Figure 6.13: The mean catch-per-unit-effort (CPUE) (fish per 1000 m^2) recorded during the study period and CPUE recorded during low DO events that occurred in February 2018, March 2018, May 2018 and October 2018 for *Glossogobius callidus* in the Swartkops Estuary during the sampling period (February 2018 – September 2019).

6.4 Discussion

In this study, four low DO events were recorded in both estuaries. In the Sundays Estuary, the lowest DO was consistently recorded in the middle reaches of the estuary, with hypoxic conditions < 2 mg/l and supersaturated conditions indicative of harmful algal blooms (HABs) (DO > 10 mg/l) recorded at a single site (Site S5) in the middle reaches during summer and autumn, respectively. In the Swartkops Estuary, four low DO events were also recorded during the sampling period, with the lowest DO (< 3 mg/l) recorded in the uppermost site (Site 7) in spring. These low DO events resulted in a decrease in fish species richness and overall abundance in the middle reaches of the Sundays Estuary, while in the Swartkops Estuary fish species richness declined during low DO events with little effect on the abundance. Species-specific responses of dominant demersal fish were observed, with all the dominant species analysed having lower CPUE in sites and months with hypoxic conditions (< 1 mg/l) and supersaturated conditions (DO > 10 mg/l) which occurred in the Sundays Estuary. Although some species did not avoid low DO events (< 4 mg/l) in both estuaries, a decrease in abundance of species was observed in affected sites.

Low DO and hypoxic conditions in bottom waters associated with high temperatures during summer is a common occurrence in many estuaries (e.g. Kemp et al. 2009; Li et al. 2018; Li et al. 2020; Coogan et al. 2021). Low DO conditions have historically also been recorded in the middle reaches of the Sundays Estuary and the upper reaches of the Swartkops Estuary during summer/spring months (Lemley et al. 2017; Adams et al. 2019). These conditions were largely attributed to increased temperature, phytoplankton biomass, and water residence time, which were identified as the important drivers that facilitate seasonal spring/summer bottom-water hypoxia (Lemley et al. 2018). The increased frequency of these events in the polluted Swartkops and eutrophic Sundays estuarine systems highlights the importance of understanding the response of demersal fishes to these low DO events.

The overall decline observed in fish abundance and species richness at Site S5 associated with the low DO events, and particularly the hypoxic event in the Sundays Estuary in February 2019, are consistent with other studies on demersal species that have shown similar declines in abundance and fish species richness triggered by low DO and hypoxic events (e.g. Craig and Crowder 2005; Eby et al. 2005; Roberts et al. 2009). Maes et al. (1998), for example, found fish species diversity and abundances declining with decreasing oxygen concentrations in the

Zeeschelde Estuary, Belgium. In the north-western Gulf of Mexico continental shelf, a decrease in species richness and total abundance occurred in low DO waters (< 4 mg/l), with an increase in fish species richness and abundance in areas where DO levels were higher (> 4 mg/l) (Craig and Bosman 2013). These effects were more severe for the benthic fish assemblage than for the pelagic assemblage in this region, as low DO is mostly limited to the bottom waters (e.g. Mcatchie et al. 2010; Craig and Bosman 2013). As such, the spatial distribution of benthic fish assemblages was altered during hypoxic condictions (Craig and Bosman 2013). Rabalais et al. (2010) suggested that declines in fish abundance and richness during low DO may be attributed to fish avoidance of the hypoxic bottom layer by either swimming upward to water above the oxycline or horizontally away from the hypoxic area or a combination of both upward and horizontal movement.

Hypoxic conditions can potentially reduce overall habitat availability (habitat squeeze) for demersal fishes by both concentrating populations into smaller geographic areas and by forcing individuals into less suitable habitats (Craig and Crowder 2005; Switzer et al. 2009). Hypoxic conditions in the Sundays Estuary resulted in zero catches at Site S5 when DO decreased to < 1 mg/l, with fish occurring at the adjacent sites with low DO. Similarly, Campbell and Rice (2014) in the Neuse River Estuary, North Carolina, USA showed that demersal fish species avoid affected areas, with fish occupying the adjacent higher DO areas. Craig (2012) in the Northern Gulf of Mexico also found that many demersal fish species migrate from the low DO areas (< 2 mg/l) and aggregate near the edge of the hypoxic zone. Other researchers also found that fish move away from hypoxic areas and occupy the adjacent higher DO zones in estuarine and marine environemnts (e.g. Pihl et al. 1991; Keller et al. 2010). This movement behaviour may alter the spatial distribution of these demersal fish species resulting in increased density in the hypoxic edges where more inter- and intra-species interactions could take place (Campbell and Rice 2014).

The response of fishes to low DO is species-specific, with many species exhibiting a negative response, while others are less affected by low DO conditions (Pihl et al. 1991; Breitburg et al. 1994; Wannamaker and Rice 2000). In the present study, despite avoidance of hypoxic waters (< 2 mg/l) by the dominant species analysed, low oxygen concentration (2 – 3.9 mg/l) had little effect on some of the demersal fish species in both estuaries. Among the set of dominant species analysed, *R. holubi* (in both estuaries), *C. gilchristi* and *G. callidus* (in the Swartkops Estuary) were not particularly affected by moderately low DO (2 – 4 mg/l), although *R. holubi* avoided

hypoxic (0.5 mg/l) areas in the Sundays Estuary. This is similar to the findings of Pihl et al. (1992) in the lower York River, Chesapeake Bay USA, where some demersal fish species remained in low DO waters (2 – 3 mg/l) but avoided hypoxic waters (< 2 mg/l). According to Pihl et al. (1992), some species remain in low DO for a short period of time to take advantage of benthic prey, which have reduced their burial depth. For example, some species such as sea nettles (Pelagiidae, *Chrysaora quinquecirrha*), naked gobies (Gobiidae *Gobiosoma bosc*) and flatfish hogchokers (Achiridae, *Trinectes maculatus*) were reported to be more tolerant of moderately low DO and were shown to survive well in concentrations of just 2 mg/l as they do not immediately move to oxygenated areas to avoid these conditions (Pihl et al. 1991; Breitburg et al. 1994). Pihl et al. (1991) related the survival of some species (e.g. *Trinectes maculatus*) exposed to low DO to physiological adaptation where ventilation rates were observed to increase up to three-fold under laboratory experiments. The increased ventilation surface area and reduced energy demand have also been reported for other demersal fishes in low DO areas (e.g. Friedman et al. 2012). These mechanisms may explain the occurrence of certain species observed in the present study in sites with low DO conditions. Numerous studies have also demonstrated that some organisms tolerate short-term exposure to low DO in order to access prey items (due to the presence of some zooplankton species which some fish species can feed on with no detrimental effect) (e.g. Taylor et al. 2007, Craig et al. 2010). For example, Switzer et al. (2009) in the nearshore marine environment of the northern Gulf of Mexico also found that some benthic species were able to survive low DO areas as these species were opportunistically feeding on weakened or recovering benthic organisms. Switzer et al. (2015) said that it should also be noted that the occurrence of some species collected during these events could be partially attributable to increased catchability of individuals under physiological stress (weakened movement or recovering organisms).

Although low DO affects fish species composition and abundance directly or indirectly, understanding avoidance behaviour requires detailed information on the behavioural responses of individuals to various environmental factors. The differences in low oxygen avoidances across the various species probably reflects the broad differences in adaptations to cope with low oxygen conditions among demersal fishes, which span a broad range of behavioural and metabolic changes (Vaquer-Sunyer and Duarte 2008). Therefore, to improve our understanding of the occurrence of some mobile species in low DO waters and to determine the impacts these may cause, laboratory and field studies need to identify the behavioural mechanisms that these

species use to avoid hypoxia and also include other environmental factors that affect avoidance behaviour in estuaries vulnerable to low DO conditions.

The results from this study nonetheless provide much needed insights into the impact of low DO and hypoxic conditions on the spatial distribution of abundant demersal fish species. The results suggest that the frequent occurrence of low DO coupled with HABs in these systems will ultimately have a major impact on demersal species occurring in the Sundays and Swartkops estuaries and the availability of quality nursery habitat. Since these estuaries still continue to support high abundances of juvenile fishes (Chapter Four), and are considered valuable nurseries (Chapter Five), determining avoidance responses to hypoxia is important for identifying species most susceptible to the direct and indirect impacts of these events. This is important for developing more ecosystem-based approaches for management of estuary-associated species.

CHAPTER SEVEN

General Discussion

Assessing fish distribution and abundance across estuarine and marine nearshore habitats is vital to the identification of ecologically important habitats and the development of effective management strategies for coastal fish populations, many of which are important fishery species. This study represents one of the few studies, particularly in South Africa, that focussed on fish community patterns, including early life history stages, across an estuary and the marine nearshore environments concurrently in order to determine the nursery value of both environments and to examine whether these two coastal environments have distinct fish assemblages or comprise a heterogeneous community organised along one or more environmental gradients (ecocline model).

Studies globally which have adopted a seascape/ecotone approach have emphasised the importance of both marine nearshore and estuarine environments as settlement and nursery habitat for coastal fish species (e.g. Blaber et al. 1995; Able et al. 2006; Woodland et al. 2012; Guerreiro et al. 2021). Although studies have highlighted much higher densities of fishes in estuaries than the adjacent nearshore marine environment (e.g. Valesini et al. 1997; Potter et al. 2000; Woodland et al. 2012; Miro et al. 2020) no studies have compared the density/abundance of all early-life stages in these two environments as an indicator of nursery function. Woodland et al. (2012) suggested that the paucity of research comparing estuarine and marine nearshore habitats is due to sampling gear limitations. Using a beam trawl net, which effectively sampled all life stages of demersal fish (postflexion larvae, transformation stages, young-of-the-year, late juveniles and adults) (Chapter Five), the present study focused on one habitat type (soft-bottom) (Chapter Four) avoiding this limitation.

The results of the present study (Chapter Five) highlighted that estuaries are indeed far more productive nursery areas for demersal fish species, with the overall density of settlement stage individuals and juveniles five to six times higher in the estuarine environment than in the marine nearshore environment (Chapter Five and illustrated in Figure 7.1). In the Algoa Bay shallow water demersal seascape, both environments were dominated by early-life stages (Chapter Five, Figure 7.1), highlighting the nursery function of estuaries and marine nearshore

soft-bottom habitats, and two discrete demersal fish assemblages were identified representing the marine nearshore and the estuary (Chapter Four). The nearshore sites were dominated by species which spawn in the marine environment and are not dependent on estuaries (marine estuarine-opportunists and marine species), whilst the estuaries were dominated by estuarine spawners and marine estuarine-dependent species (Chapter Four, illustrated in Figure 7.1).

This pattern was consistent across the early life stages, with the high density of settlement, young-of-the year and late juveniles in the estuarine environment dominated by estuarine spawners and estuarine-dependent marine species (Figure 7.1), highlighting the importance of estuaries as nursery areas for demersal estuarine and marine estuarine-dependent species. These dominant estuarine-associated species (MED and E&M) are euryhaline species that are able to tolerate fluctuating environmental conditions in estuaries and make use of the abundant food resources present in these systems (discussed in Chapters Four and Five). Although the density of settlement stages and juveniles was far lower in the marine nearshore, the marine nearshore environment is an important nursery area for more stenohaline MEO, MS and M species (Figure 7.1) that may be less tolerant of the fluctuating environmental variables in the middle and upper reaches of estuaries (Whitfield 2019).

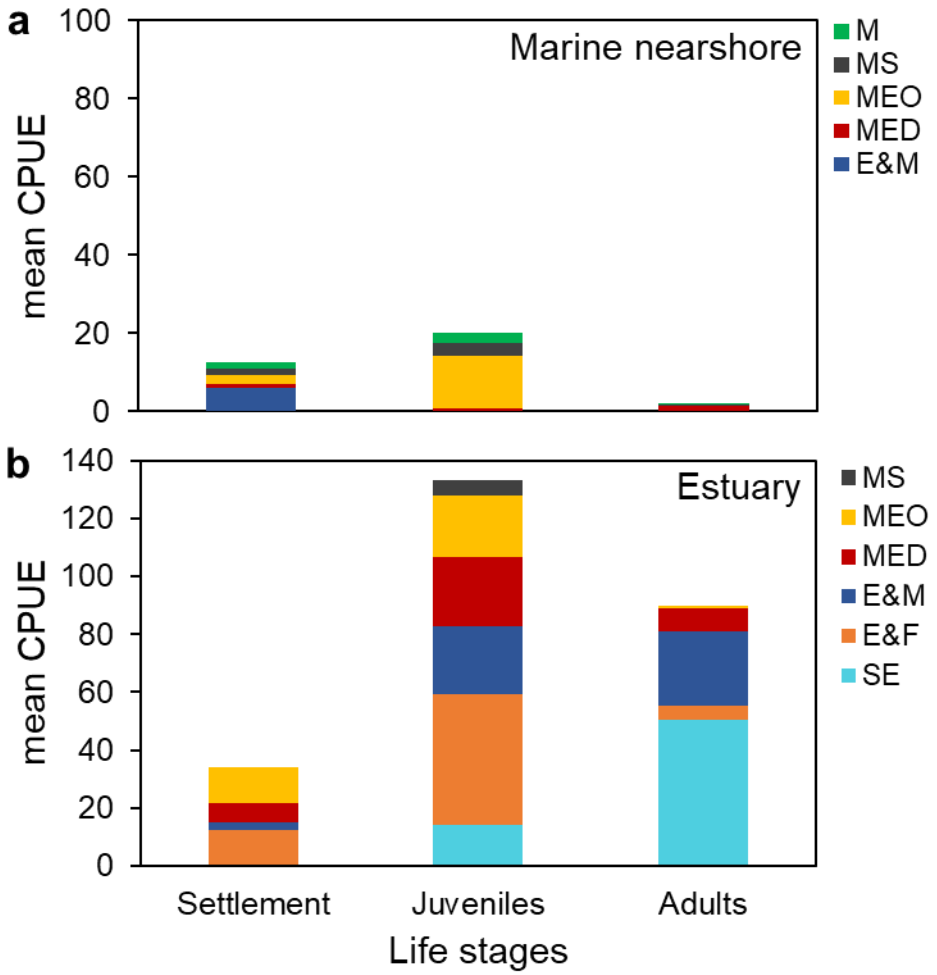

Figure 7.1: The mean catch-per-unit-effort (CPUE) (fish per 1000 m^2) of settlement stages, juveniles and adults in the (a) marine nearshore and (b) estuarine environments during the sampling period (July 2017 – September 2019). Estuarine association category: M = marine species, MS = marine stragglers, MEO = Marine estuarine-opportunist, MED = Marine estuarine-dependents, E&M = Estuarine and marine, E&F = Estuarine and freshwater species.

Many of these species form an important component of fisheries in South Africa (see Table 7.1). As such, understanding spatial patterns of abundance of demersal species across life stages and guilds is essential in identifying areas that are important as nursery habitats for conservation planning to both preserve biodiversity and ensure recruitment of juveniles to exploited adult fish stocks.

Table 7.1: Exploited fishery species recorded in Algoa Bay marine nearshore (AB), Sundays (S) and Swartkops (SW) estuaries during the sampling period (July 2017 – September 2019). IT = marine inshore trawl, RL = recreational linefishery, CL = commercial linefishery, SL = small-scale linefishery. Fisheries and stock status from Mann (2013).

Family	Exploited species	Fisheries	Locality	Stock status
	Marine and marine stragglers			
Cynoglossidae	*Cynoglossus zanzibarensis*	MIT	AB	Not assessed
	Cynoglossus capensis	MIT	AB	Not assessed
Dasyatidae	*Dasyatis chrysonota*	RL	AB	Not assessed
Sciaenidae	*Argyrosomus inodorus*	RL, CL, MIT	AB	Collapsed (<25%)
	Atractoscion aequidens	RL, CL	AB	Collapsed (<25%)
	Umbrina canariensis	CL	AB	Collapsed (<25%)
Triglidae	*Chelidonichthys kumu*	CL	AB	Least concern
	Marine estuarine-dependent			
Haemulidae	*Pomadasys commersonnii*	SL, RL	S,SW	Overexploited (25 - 40%)
Sciaenidae	*Argyrosomus japonicus*	RL, CL, MIT	S	Collapsed (<25%)
Sparidae	*Lithognathus lithognathus*	RL	SW,S	Collapsed (<25%)
	Rhabdosargus holubi	RL, SL	AB, SW, S	Not assessed
Elopidae	*Elops machnata*	RL	S	Not assessed
	Marine estuarine-opportunist			
Haemulidae	*Pomadasys olivaceus*	SL, RL	AB, S, SW	Optimally exploited (40 - 50%)
Sparidae	*Sarpa salpa*	RL, SL	AB	Not assessed
	Diplodus capensis	RL	AB, SW, S	Optimally exploited (40 - 50%)
	Spondyliosoma emarginatum	RL	AB, SW, S	Not assessed
	Rhabdosargus globiceps	MIT	AB, SW, S	Not assessed
Platycephalidae	*Platycephalus indicus*	RL	SW, S	Not assessed

Algoa Bay has been chosen as a case study for the first South African Marine Spatial Plan (MSP) (Dorrington et al. 2018). One of the main aims of the initial phase of this plan is to map patterns of biodiversity, species composition, distribution and abundance (Dorrington et al. 2018). The outcomes from the current study directly address this aim for demersal fish species in the Algoa Bay seascape.

In terms of mapping overall demersal fish assemblages (abundance and distribution patterns) within the Algoa Bay seascape, hotspot nursery regions for settlement and juvenile demersal fishes were identified (Chapter Five) and depicted spatially by life stage in Figure 7.2. In the upper reaches of the Swartkops Estuary density of these stages ranged from 70 to 160 fish per 1000 m^2 compared to the marine nearshore environment, where these early-life stages ranged from 0.1 to 10 fish per 1000 m^2 (Figure 7.2). In the Sundays Estuary, much higher densities of

settlement and juvenile stages were recorded in the lower reaches ranging between 40 and 110 fish per 1000 m^2 (Figure 7.2).

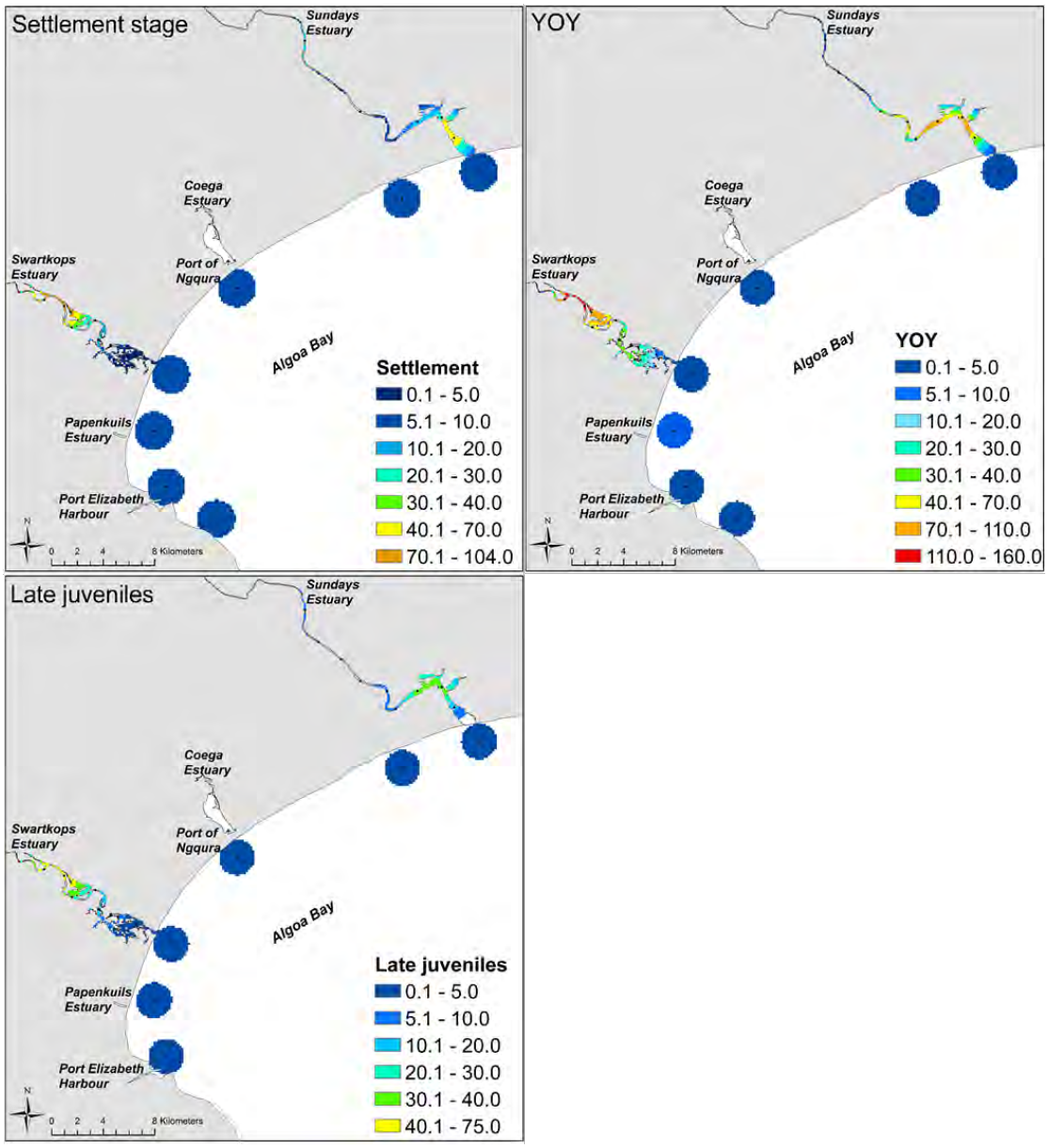

Figure 7.2: Density (mean catch-per-unit-effort (CPUE) (fish per 1000 m^2)) of settlement stages, young-of-the-year (YOY) and late juveniles for demersal fishes in each site in the marine nearshore, Sundays and Swartkops estuaries during the sampling period (July 2017 – September 2019).

The density hotspots in the upper reaches of the Swartkops and lower reaches of the Sundays Estuary were comprised of MED and E&M settlement, YOY and late juvenile stages (Figure 7.3). Settlement and juvenile stages of M and MS were recorded in the marine nearshore, with

higher densities near the Swartkops Estuary mouth and the Papenkuils Outlet (Figure 7.4). Marine estuarine opportunist (MEO) species were caught in the lower reaches of both estuaries and the marine nearshore, with higher densities of late juveniles recorded in the marine nearshore, also around both estuary mouths and the Papenkuils Outlet (Figure 7.4).

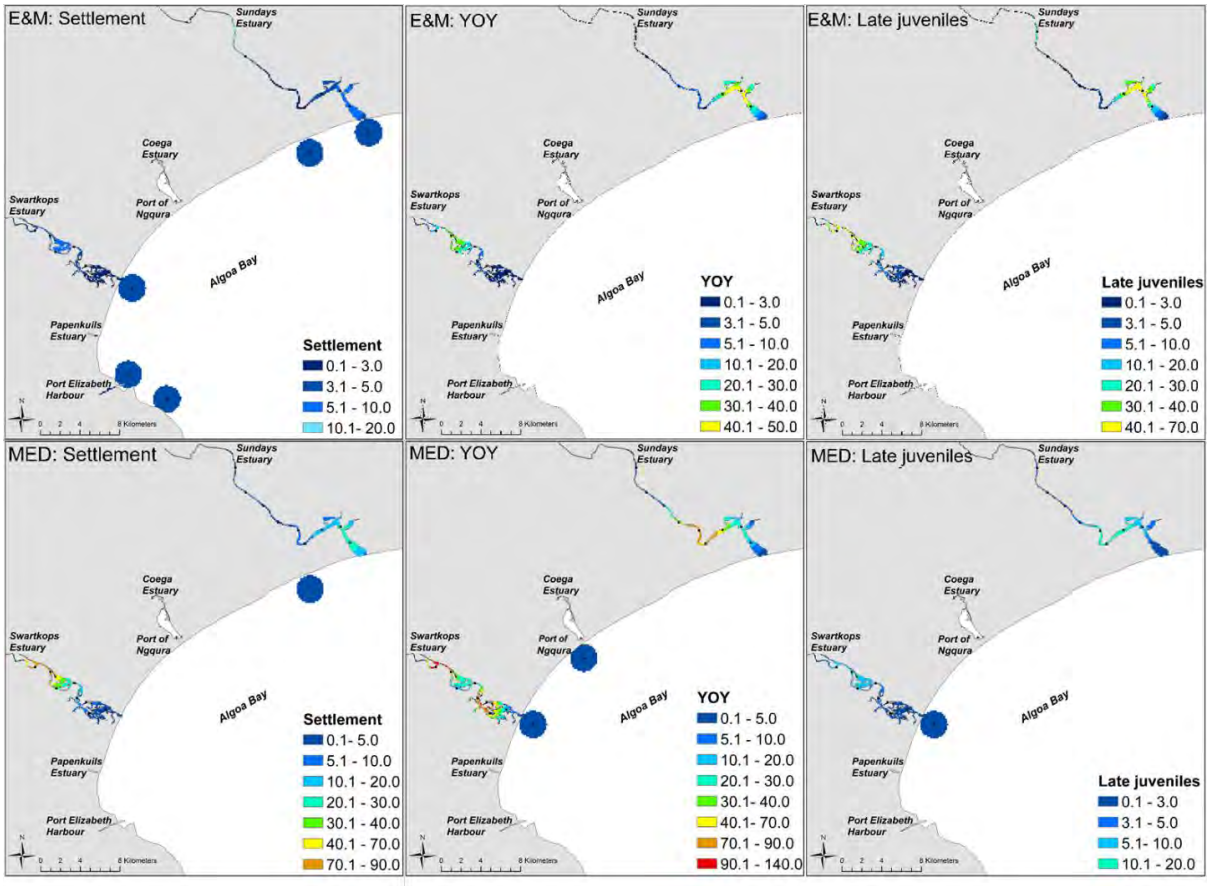

Figure 7.3: Density (mean catch-per-unit-effort (CPUE) (fish per 1000 m^2)) of settlement stages, young-of-the-year (YOY) and late juveniles for estuarine and marine (E&M) and marine estuarine-dependent (MED) in each site in the marine nearshore, Sundays and Swartkops estuaries during the sampling period (July 2017 – September 2019).

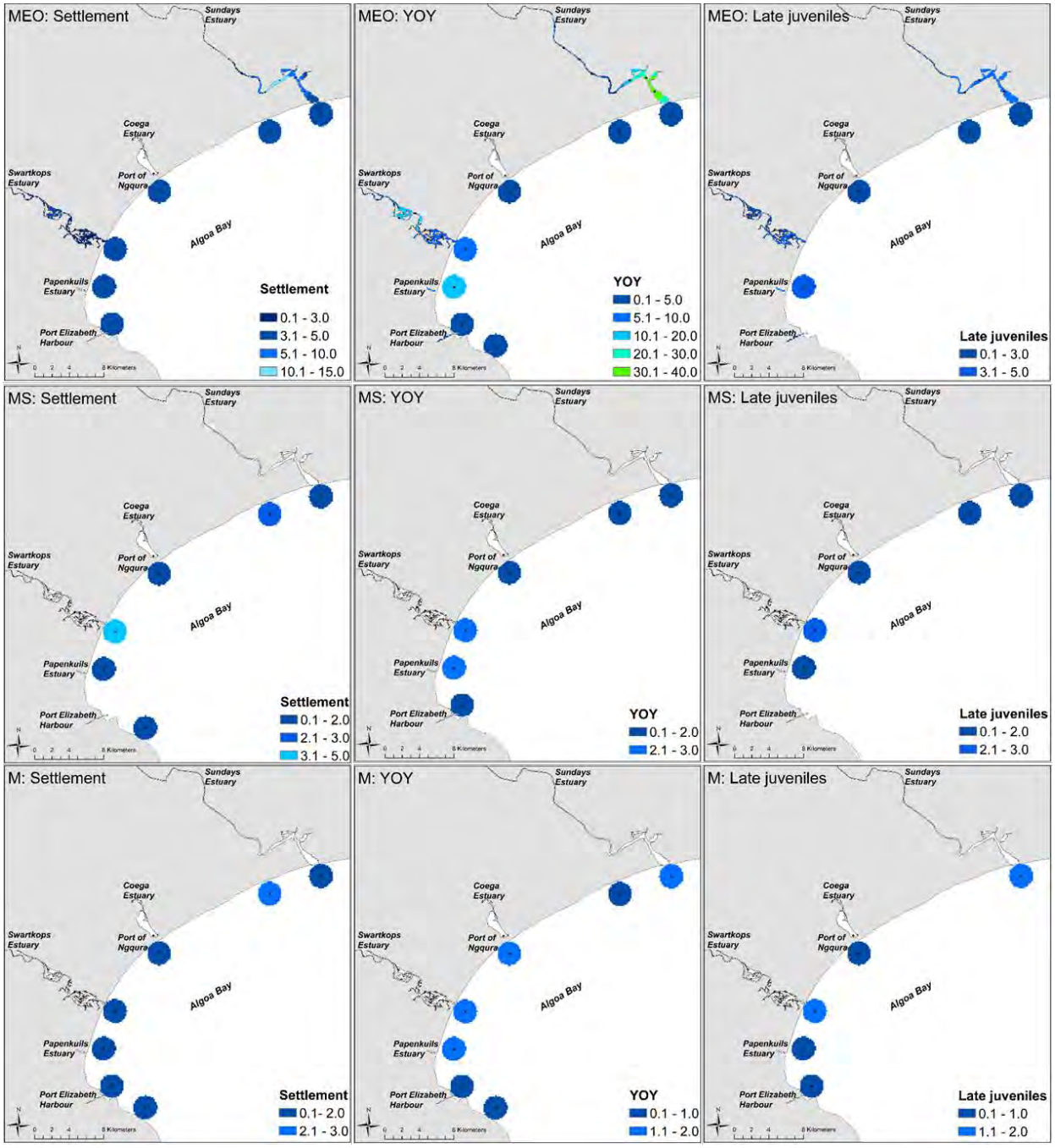

Figure 7.4: Density (mean catch-per-unit-effort (CPUE) (fish per 1000 m^2)) of settlement stages, young-of-the-year (YOY) and late juveniles for marine estuarine-opportunists (MEO), marine stragglers (MS) and marine species (M) recorded in each site in the marine nearshore, Sundays and Swartkops estuaries during the sampling period (July 2017 – September 2019).

The greater density of settlement and juvenile marine species near estuarine mouths and outlets in the marine nearshore environment is related to freshwater inflow and needs to be highlighted in the MSP as well as estuarine management plans to ensure adequate supply of freshwater to the marine environment. Freshwater flow into marine nearshore areas influences coastal fish

and fisheries abundance through its effects on primary productivity (Gillanders and Kingsford 2002). Lamberth et al. (2009) also highlighted the importance of sufficient freshwater flow into the marine nearshore environment for marine species. In marine nearshore waters of KwaZulu-Natal on the east coast of South Africa many non-estuarine associated marine species increased in abundance after high flow from estuaries.

Altered freshwater flows and consequent variations in salinity can have an impact on the nursery use of estuaries by marine estuarine-dependent species (Nodo et al. 2018). For example, Nodo et al. (2018) in the freshwater-deprived Kariega Estuary, South Africa, noted a significant increase in the marine estuarine-dependent species *P. commersonnii* and *A. japonicus* after a flood event that resulted in 'normal' estuarine conditions relative to the previous studies when there was little or no freshwater input. The supply of freshwater into estuaries is vital for the sustainability of these important fishery species and also needs to be incorporated into estuarine management plans.

Increasing fishing pressure in coastal nursery areas, as well as inadequate management of the coastal regions, has caused many exploited fishery species to be more susceptible to overexploitation (Lamberth and Turpie 2003). This has resulted in several stocks of many exploited species in South Africa collapsing (Turpie et al. 2002; Lombard et al. 2004; Potts et al. 2020; DEFF 2016). Identifying settlement and nursery areas for exploited species should also feed into the MSP as well as resource and estuary management plans. Important marine estuarine-dependent exploited species *Rhabdosargus holubi*, *Pomadasys commersonnii*, *Argyrosomus japonicus* and *Lithognathus lithognathus* (see Table 7.1) were found in high numbers at the juvenile stage in the lower and middle reaches of the Sundays Estuary (Figure 7.5). The delayed maturity and dependence on estuaries as juveniles has resulted in high levels of overfishing in estuaries and a collapsed stock status of *A. japonicus* (Childs and Fennessy 2013) and over-exploited stock status of *P. commersonnii* (Cowley and Fennessy 2013).

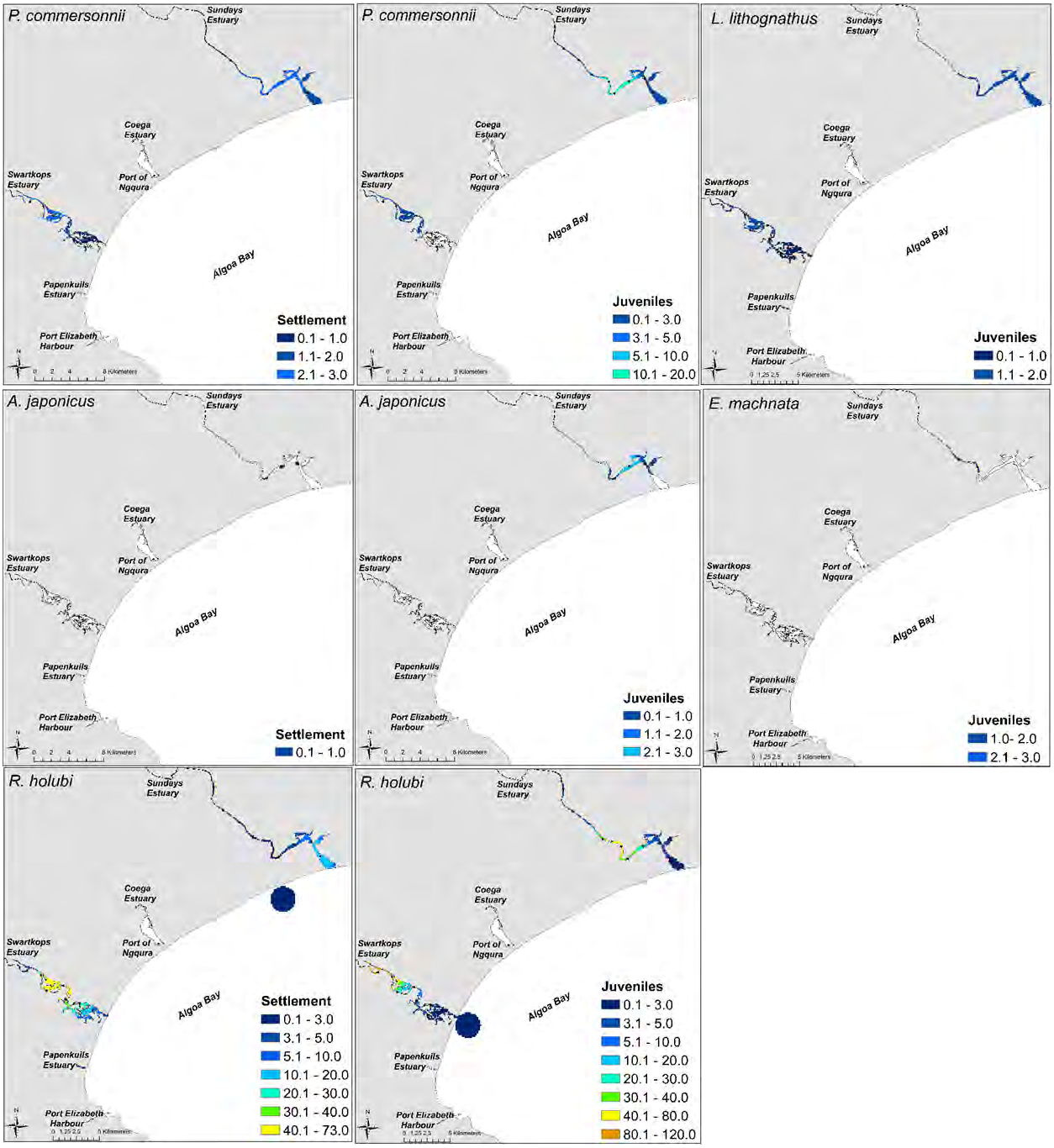

Figure 7.5: Density (mean catch-per-unit-effort (CPUE) (fish per 1000 m^2)) of the settlement stages and juveniles of exploited MED species caught in the marine nearshore, Sundays and Swartkops estuaries during the sampling period (July 2017 – September 2019).

Exploited marine species caught in the marine inshore trawl fishery include *Cynoglossus capensis*, *Cynoglossus zanzibarensis* and *Argyrosomus inodorus* (Table 7.1, Figure 7.6). The identification of the larval and settlement stage fish for some important fishery species was only possible when DNA barcoding was used as most of these species were misidentified morphologically. Marine fish species that were recorded in higher numbers near the Swartkops

Estuary mouth and the Papenkuils Outlet in marine nearshore include *Umbrina canariensis, Atractoscion aequidens, Dasyatis chrysonota, Chelidonichthys kumu* (Figure 7.6). Chalmers (2012) also identified the shallow marine nearshore of the sheltered western region of Algoa Bay (near estuary mouths) as nursery/aggregation areas for many exploited marine species (based angler interviews).

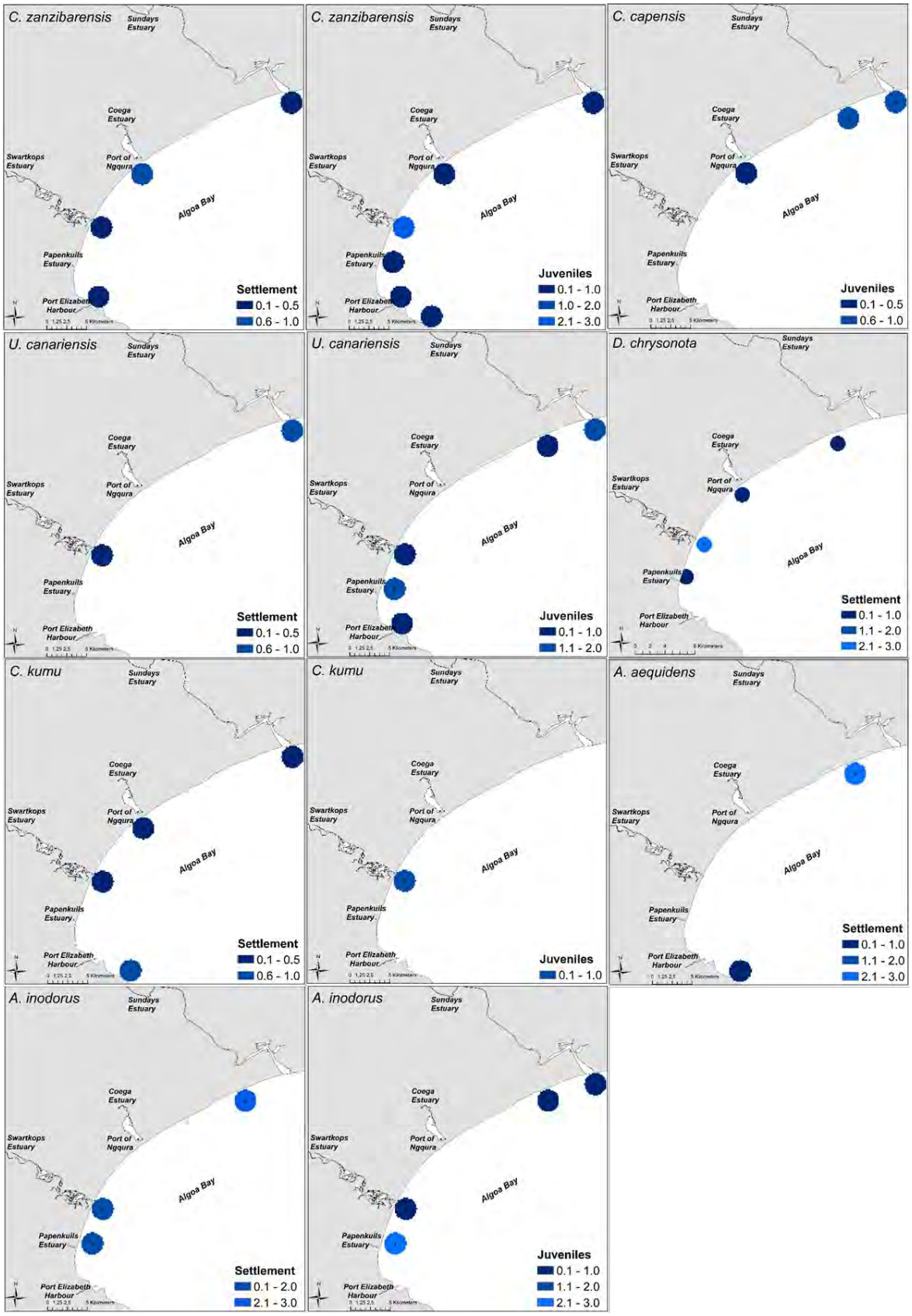

Figure 7.6: Density (mean catch-per-unit-effort (CPUE) (fish per 1000 m^2)) of the settlement stages and juveniles of exploited marine species (M) caught in the marine nearshore during the sampling period (July 2017 – September 2019).

Exploited MEO species caught in this study include *Pomadasys olivaceus, Sarpa salpa, Spondyliosoma emarginatum, Diplodus capensis* and *Rhabdosargus globiceps*. The settlement stage of morphologically similar species, such as *P. olivaceus* and *S. emarginatum,* were correctly identified by DNA barcoding as they were not easily distinguishable in the field (Chapter Three). Density hotspots for these exploited species were identified in the lower reaches of both estuaries and the marine nearshore around estuary mouths and the Papenkuils outlet (Figure 7.7).

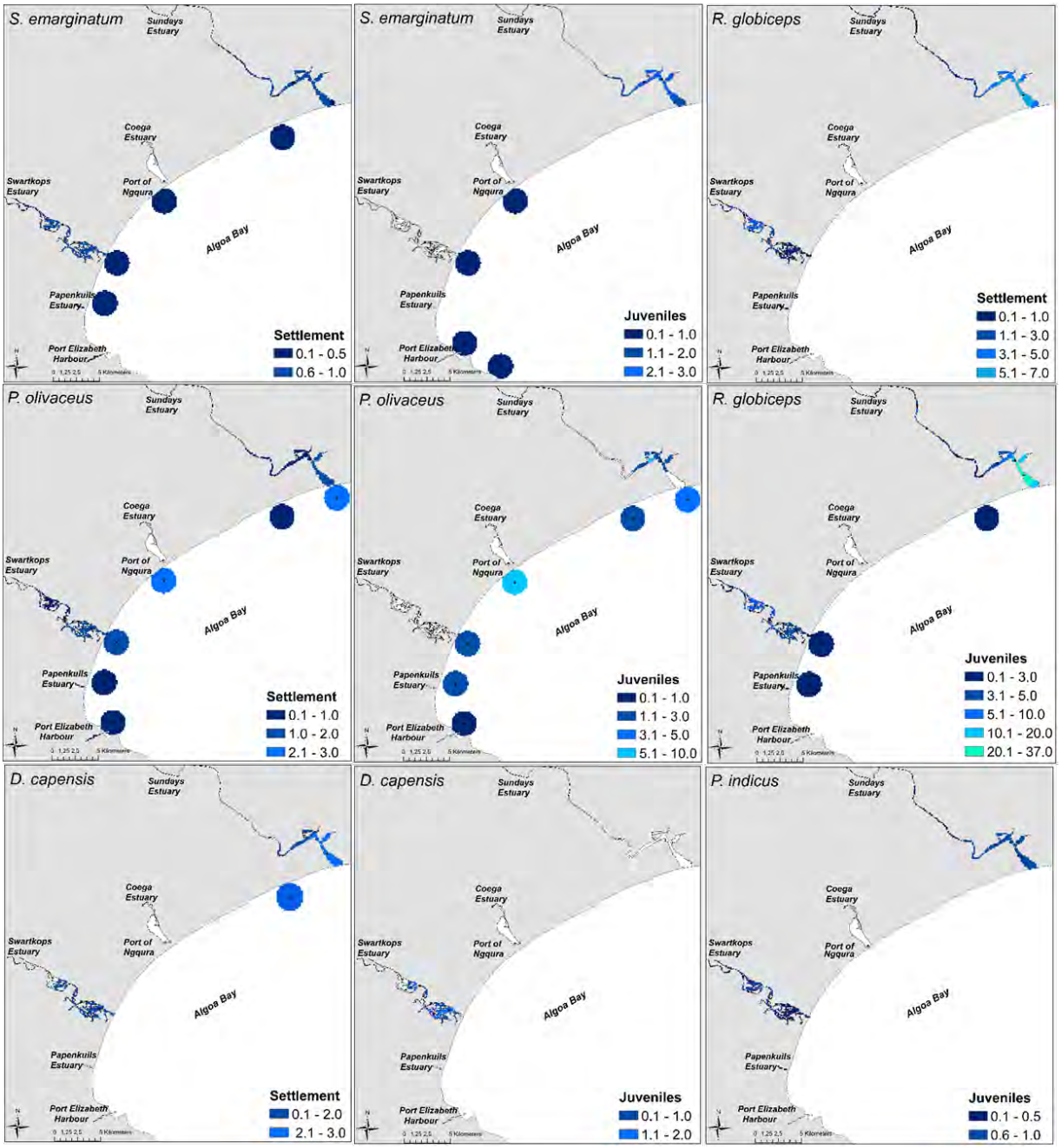

Figure 7.7: Density (mean catch-per-unit-effort (CPUE) (fish per 1000 m^2)) of settlement YOY and late juveniles for MEO species in each site in the marine nearshore, Sundays and Swartkops estuaries during the sampling period (July 2017 – September 2019).

These coastal ecosystems serve as important nursery areas, however they are facing many challenges, including climate change (e.g. droughts, storms and floods) and other anthropogenic impacts (such as freshwater abstraction, overfishing, habitat destruction and pollution) (Sink et al. 2011; Green et al. 2014). Habitat destruction (e.g. destruction of reefs, seagrass beds, saltmarshes), fishing pressure and pollution are recognized as the most important threats to coastal ecosystems (Sink et al. 2011). Hypoxia (owing to the intensification of agriculture and subsequent eutrophication) is one of the major threats to the functioning of coastal ecosystems, particularly estuaries. The results from this study provide much-needed insights into the impact of low dissolved oxygen (DO) and hypoxic conditions on the spatial distribution of the abundant demersal fish species. Both the upper reaches of the Swartkops Estuary and middle reaches of the Sundays Estuary have been identified as important nursery areas for important estuarine-dependent fishery species, the occurrence of these low DO, and hypoxic conditions in these areas will have a detrimental effect on the nursery functioning of these systems. Therefore, managers must focus on protecting these nursery areas and prevent further occurrence of these low DO and hypoxic conditions caused by high nutrient loading. This can be done by incorporating these regions into the current estuarine management and restoration plans.

This study provides information on patterns of biodiversity, species composition, distribution and abundance of demersal fish in the Algoa Bay shallow water seascape which can be incorporated into the Algoa Bay MSP as well as estuarine and resource management plans. Areas identified as nursery habitats for these ecologically and economically important species should be prioritised in the management of these coastal ecosystems. As the Algoa Bay shallow water demersal seascape is located in a densely populated area any future spatial conservation networks must be assessed for socio-economic implications and the livelihoods dependent on them.

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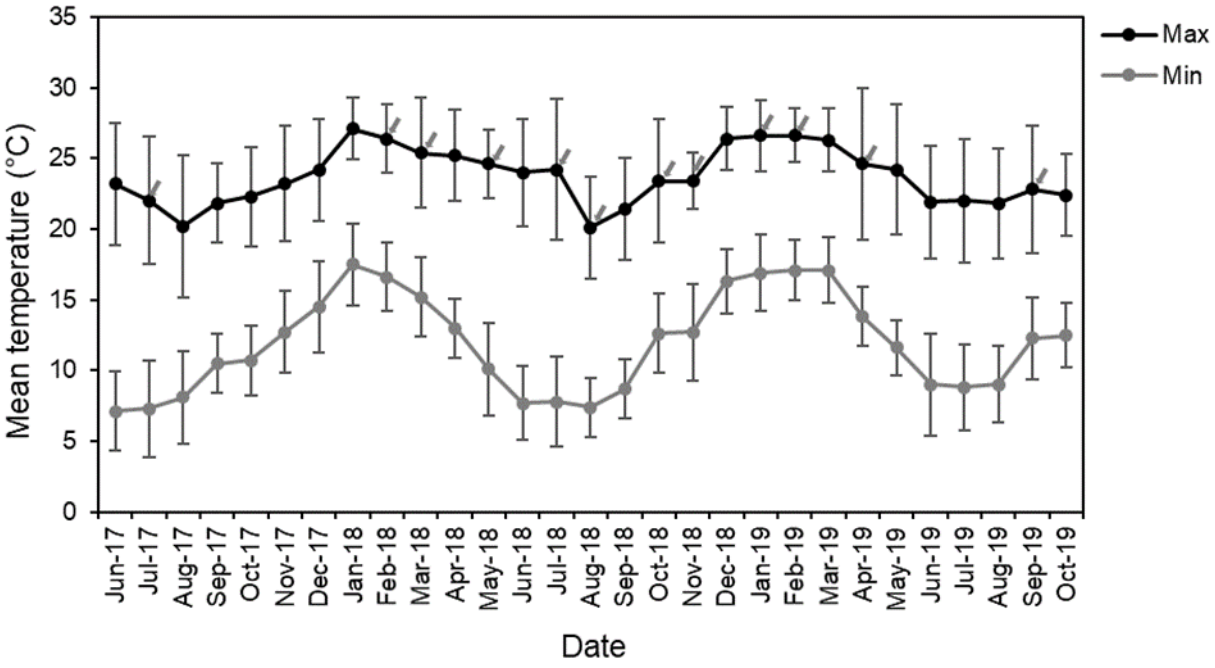
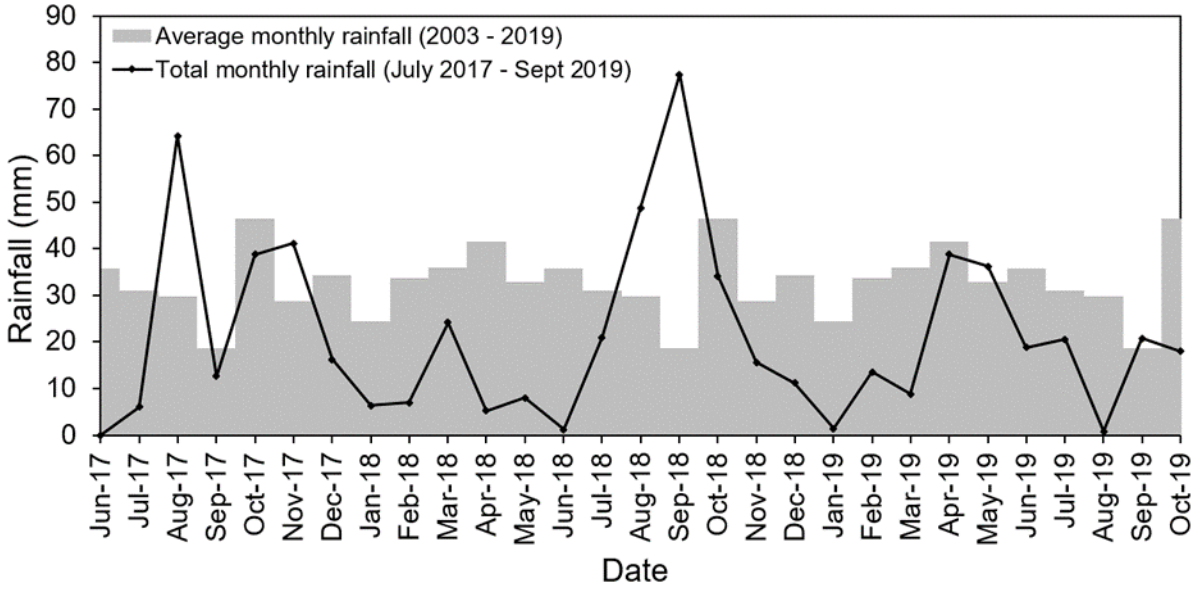
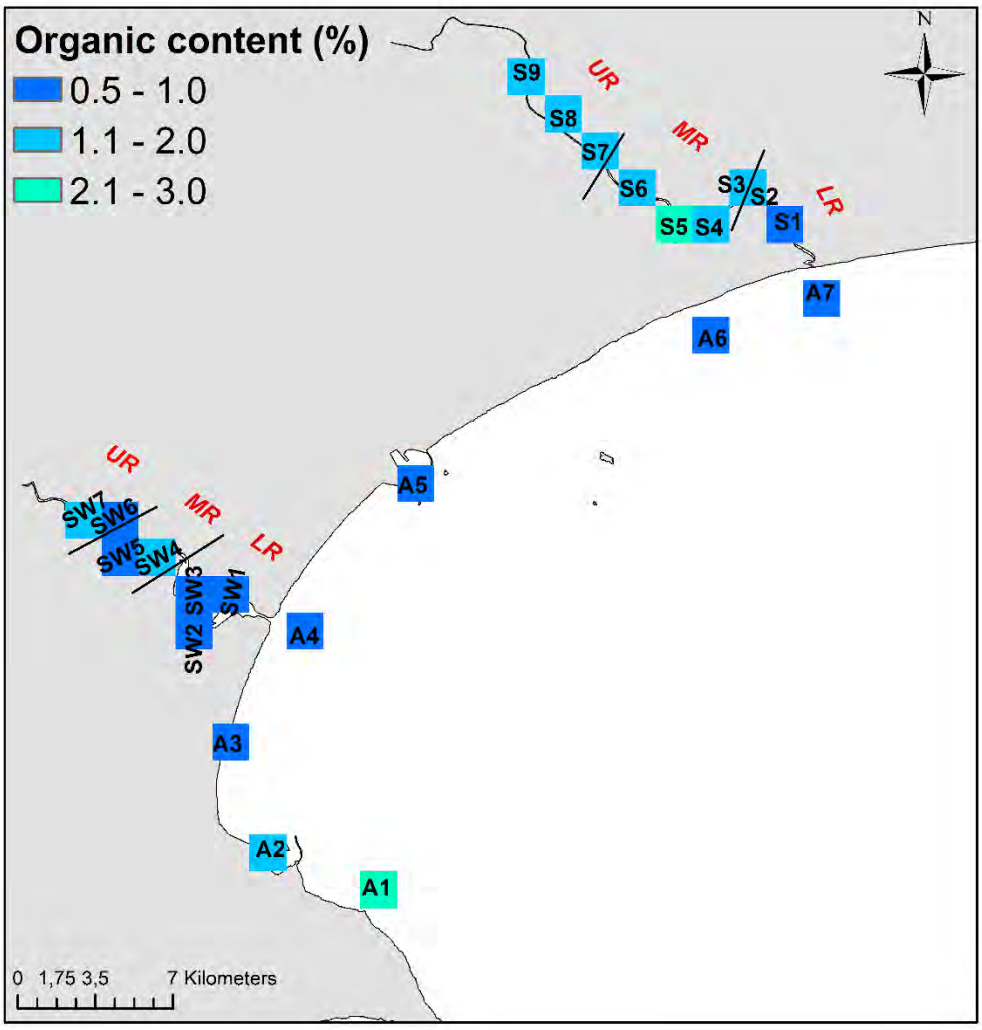
Appendix



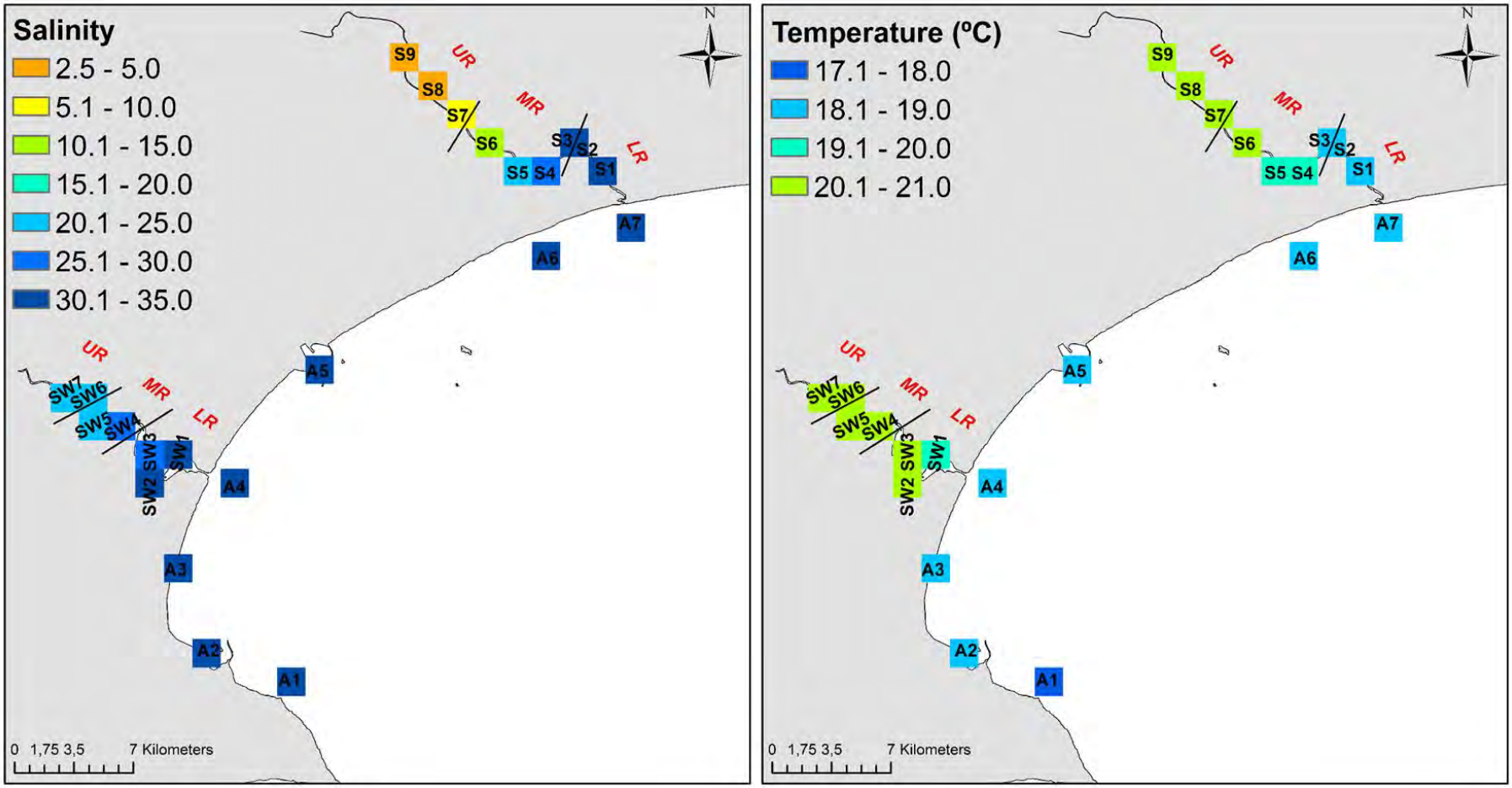
Appendix 2.1. The mean (±SD) monthly air temperatures (°C) (minimum and maximum) measured at Port of Ngqura during the sampling period (July 2017 – September 2019) Grey arrows indicate sampling dates. The data were obtained from South African Weather Service, Port of Ngqura weather station (South African Weather Bureau).



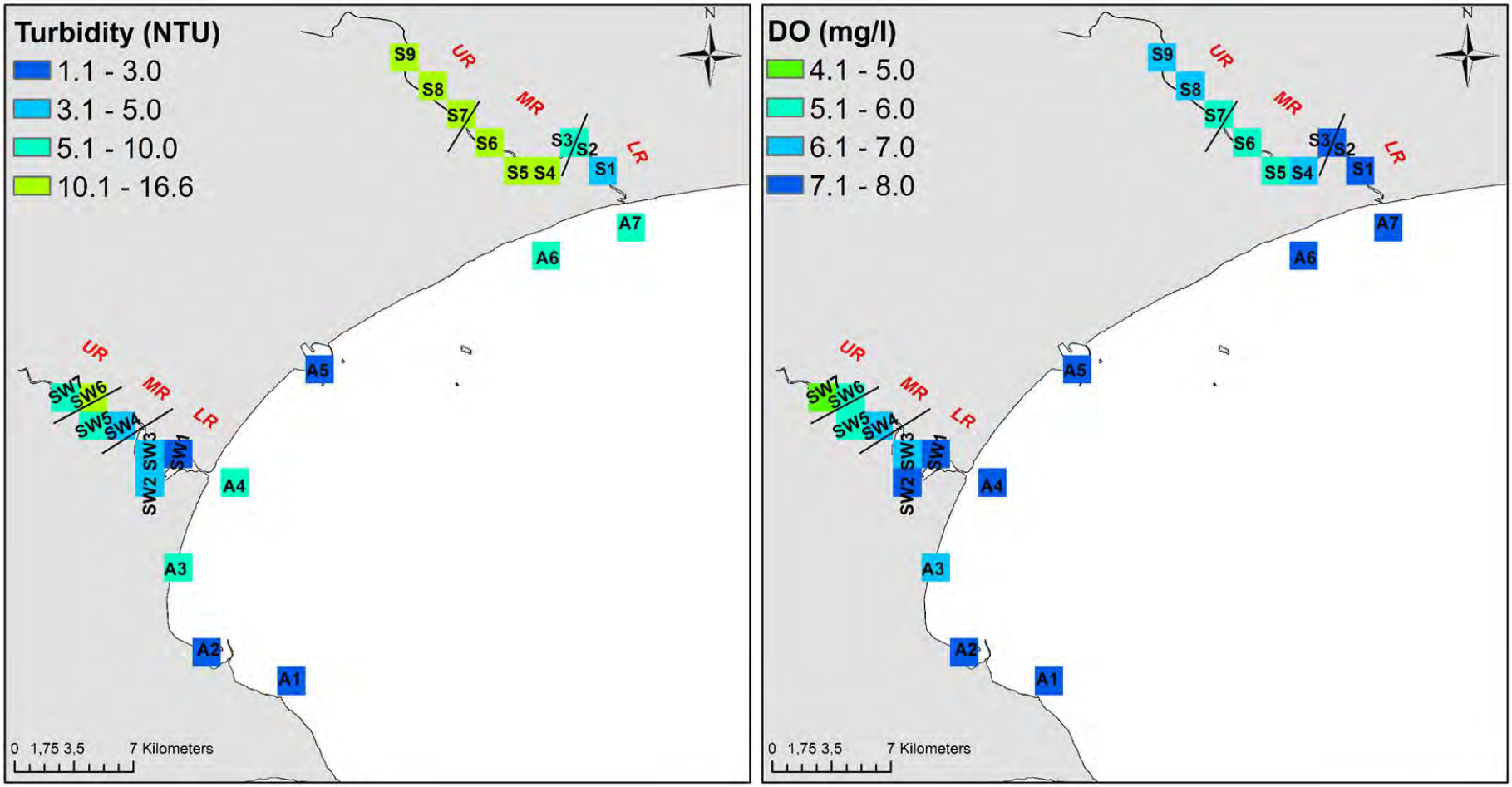
Appendix 2.2. Monthly rainfall recorded at the Port of Ngqura in Algoa Bay during the study period (July 2017 – September 2019) plotted against the average monthly rainfall from 2003-2019. Data were obtained from the South African Weather Service (SAWS).



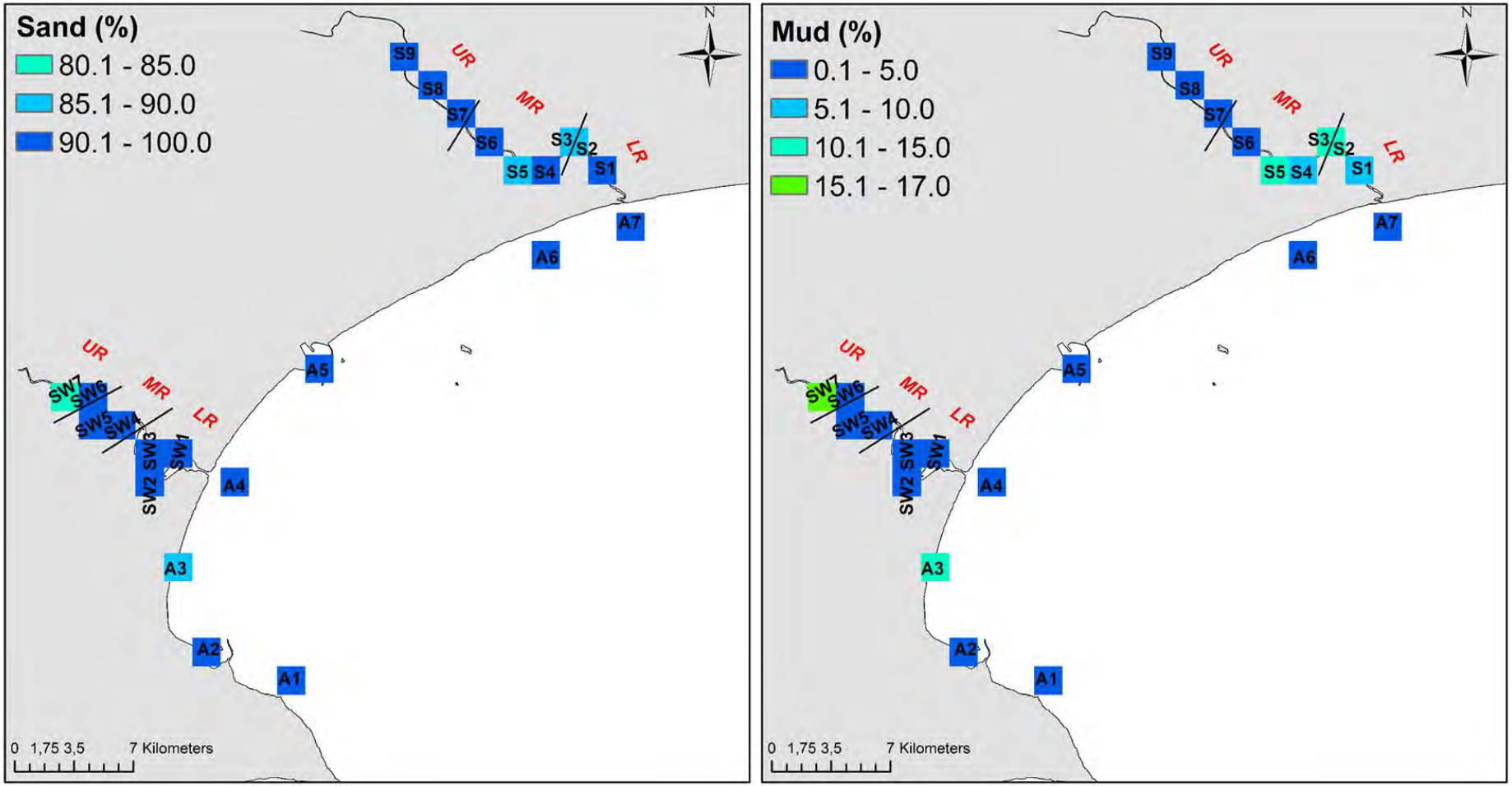
Appendix 2.3. Mean organic content in each site in the Algoa Bay marine nearshore, Sundays and Swartkops estuaries during the sampling period (July 2017 – September 2019).



Appendix 2.4. Mean salinity and temperature (ºC) in each site in the Algoa Bay marine nearshore, Sundays and Swartkops estuaries during the sampling period (July 2017 – September 2019).



Appendix 2.5. Mean turbidity (NTU) and dissolved oxygen (DO) (mg/l) in each site in the Algoa Bay marine nearshore, Sundays and Swartkops estuaries during the sampling period (July 2017 – September 2019**)**.



Appendix 2.6. The percentage of the sediment particle size, sand and mud in each site in the Algoa Bay marine nearshore environment, and the Sundays and Swartkops estuaries during the sampling period (February)