Spatial analysis of littoral and demersal fish assemblages within the Knysna Estuary system

Thesis submitted in fulfilment of the requirement for the degree of

MASTER OF SCIENCE

of

RHODES UNIVERSITY

by

Andrew Meiklejohn

February 2023

ABSTRACT

The Knysna Estuary is a unique system as it is the only "estuarine bay" system in the warm-temperate region of South Africa and is the largest estuary system on the southern coastline of South Africa. The Knysna Estuary has been identified as the estuary with the highest conversation priority in South Africa. The volume of research undertaken on the Knysna Estuary has led to it being rated "excellent" in terms of research productivity. However, despite this, surprisingly little work has been undertaken and published around the dynamics of fish assemblages occurring in the system, with little to no research assessing the demersal fish assemblage. The last widespread fish sampling effort that has been published was conducted in 1994, highlighting the need for an updated fish assessment with a focused sampling effort targeting both the littoral and demersal fish populations. The identification of key habitats for estuarine fish assemblages is essential for addressing estuarine conservation needs. Despite the importance of spatial data in addressing conservation planning, few estuarine studies have used spatial analyses in Geographic Information Systems (GIS) to identify conservation priority areas. Such information is critical for effective estuarine management plans.

The aim of this project was to identify juvenile fish density hotspots to inform future systematic conservation planning. This study made use of two methods of sampling, seine netting to target the littoral fish assemblage and beam trawling to target demersal fish species. Two dedicated sampling trips for each sampling method were undertaken in June 2021 and March 2022 for Seine net sampling and November 2021 and March 2022 for Beam trawl sampling. Fish were identified to species level, measured (mm TL) and categorised into life history stages (i.e. settlement stage, juvenile and adult) and thereafter assigned into their various estuarine association guilds.

A total of 47 species were recorded, with 42 species were caught in the seine net sampling and 24 species in the beam trawl sampling. The results from this study showed the vast extent of marine dominance in the Knysna Estuary, with marine estuarine-opportunist (MEO) species dominating catches (richness) from both sampling methods. The spatial analysis highlighted the importance of the lower “marine bay” region of the system, with the majority of estuarine guilds showing a high abundance in this region.

Key fishery species and dominant fish species were identified during this study, the dominant species during the sampling effort were identified as ecologically important fish species and were dominated by adult specimens. The key fishery species sampled during this study were dominated by juvenile specimens. This highlights the role of the Knysna Estuary as a nursery area for juvenile fishery species and the contribution of the estuary to the estuarine and adjacent coastal fisheries. Key hotspots were identified for fishery species, these being the “Ashmead Channel” in the marine bay region and the “Belvidere” section of the lagoon region. Ashmead channel is sheltered backwater area while the Belvidere section is some distance from the main river channel, reducing the anthropogenic impact on these areas. The low anthropogenic utilization of these areas along with weaker water current in these regions was linked to the usage of these region by key fishery species. These areas were highlighted as important conservation hotspots with both currently not adequately protected under the current habitat sensitivity management model.

Key words: estuarine; ecology; spatial analysis; juvenile hotspots; demersal fish

PREFACE

Declaration

I, Andrew Meiklejohn, 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 A-R Childs, Mr K Smith, Prof NC James, Prof KI Meiklejohn and Dr P Nodo. This thesis has not been submitted to any other university.

Signature: 

Ethics statement

The sampling which forms part of this thesis, received research ethical approval from the Rhodes University Animal Research Ethics Committee under the project title "GIS analysis of spatial and temporal fish assemblages within the Knysna Estuary system" under the application number 2021-5213-6426.

South African National Park research agreement

The sampling which forms part of this thesis took place within the Knysna section of the Garden Route National Park and therefore a research agreement was required to be approved prior to sampling activities. The application MIEK-A/2021-22 SS382 was approved, and research activities were undergone in accordance with SANParks Standard Operating Procedure.

ACKNOWLEDGEMENTS

I would like to thank my supervisor team; Prof A-R Childs, Mr K Smith, Prof NC James, Prof KI Meiklejohn and Dr P Nodo, for providing me with support and guidance throughout my masters. A huge thanks to Amber for always being available to help me and provide me with guidance throughout the masters no matter how busy you were, your guidance and patience with my subpar writing was a massive help. Thank you to Kyle for the guidance in the field and massive assistance during the writing up of this project, your guidance on sampling especially when I was new to field work was a huge help and meant the field work for this project went about as smoothly as possible. To Nikki a massive thanks for all the assistance with the writing up of this project, your expertise and willingness to help were hugely appreciated. A very special thanks to must go to my dad for the support provided throughout the masters when I was struggling and for the assistance with the GIS mapping that was critical for this project, these incredible maps were all thanks to you. Thank you to Phakama for the assistance in the field, without you I would probably still be on the river trying to identify different clinids, to have such an expert helping me in the field really made a massive difference.

This project would not have been possible without the funding provided by the Thesen Island Homeowners Association and the Rhodes University Research Grant. A big thanks must go to the Thesen Island Homeowners and the Knysna Basin project for their support and hospitality throughout this project's duration.

A massive thanks to all those who assisted in the field work, the DIFS honours class of 2021, Aidan, Xolani and the SANParks team at Rondevlei. Your hard work in the mud, sun, wind and rain is what made all of this work possible. A big thanks to the staff at DIFS and SANParks for ensuring that I had access to the right and properly functioning equipment in the field.

To my friends and classmates who made suffering long hours in the office slightly more bearable, I will forever be grateful that I got to finish writing up with my mates. As difficult as the last few months were at least we were all in the same boat suffering together. Last but not

least I would like to thank my family for all the support through the last two years, when I was struggling, when it was going well, your encouragement and support has helped me carry on and push through to get it done.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

CHAPTER ONE

GENERAL INTRODUCTION

1.1 Estuarine importance and conservation

Estuaries act as a crucial link between freshwater and marine environments; with mixing of fresh and marine water creating a nutrient-rich environment (Burchard and Baumert 1998). Many fish species rely on estuaries where the mixing of water also creates optimal salinity and temperature conditions for the early life stages of many fish species (e.g. Vanalderweireldt et al. 2020); this leads to abundant biomass of fish species and high levels of productivity (Barletta and Costa 2009, Whitfield 2019). Fish living in estuaries do, however need to be able to tolerate a broad range of physiochemical conditions with rapid changes in temperatures, salinity, turbidity, and other environmental variables being commonplace in estuaries (Whitfield 2019). Fish able to deal with the environmental pressure associated with estuarine habitats are able to live in a productive habitat providing opportunities for rapid growth. Estuaries are habitats with higher primary productivity and increased turbidity when compared with surrounding marine habitats providing high food availability and protection for juvenile life stages of marine fish species (Kikuchi et al. 2019).

Estuaries are therefore recognised as important nurseries and feeding habitats for many fish species, holding a critical place in coastal ecosystems worldwide (Sirois and Dodson 2000, North and Houde 2003, Winkler et al. 2003, Whitfield and Cowley 2010). Estuaries provide habitats for several important commercial and recreational fishery species and other ecologically important marine fish species (Lamberth and Turpie 2003, Pradervand et al. 2003, Meynecke et al. 2008, Barletta and Costa 2009, van Niekerk et al. 2019a). Juveniles of marine fish species make use of the high productivity and protection afforded by turbid waters and vegetated habitats associated with estuaries (Thiel et al. 2003). A few species live out their entire life cycle in estuaries, with the high productivity and protection characteristic of estuaries providing suitable habitats for all life stages of these fish (Whitfield 2019).

In South Africa, estuaries are crucial for many fish species, with 155 fish species being frequently recorded in these estuaries (Whitfield 2019). A hundred and three of these species (66%) are either entirely or partially dependent on the estuaries, using them as breeding grounds or nurseries and foraging areas (Whitfield 2019). Marine species show various degrees of reliance on estuarine systems, with some species making opportunist use of estuaries when conditions are best suited, while other species are completely reliant on estuaries for juvenile life stages (Whitfield 2019). Estuaries, therefore, play a vital role in the survival and protection of many of South Africa's coastal fish species. Many marine species are reliant on estuaries as a nursery habitat, with over 61 species of the 155 estuarine-associated fish species in South Africa making use of estuaries as nursery habitats in some way (Whitfield 1994). Whitfield (2000) found that 68% to 75% of the species richness in South African estuaries is made up of marine taxa, between 10% and 25% is made up of resident estuarine species, while freshwater species made up less than 7% of species richness.

The South African coastline is made up of three unique biogeographic regions, the subtropical region, which runs from the Mozambique border at Kosi Bay to the Mdumbi Estuary along the Wild Coast; the warm-temperate region, which runs to Cape Agulhas and the cool-temperate region, which runs to the Namibian border at the Orange River (Figure 1.1) (Harrison 2002). There are an estimated 127 subtropical estuaries, 127 warm-temperate estuaries, and 26 cool-temperate estuaries along the South African coastline (Whitfield and Baliwe 2013).

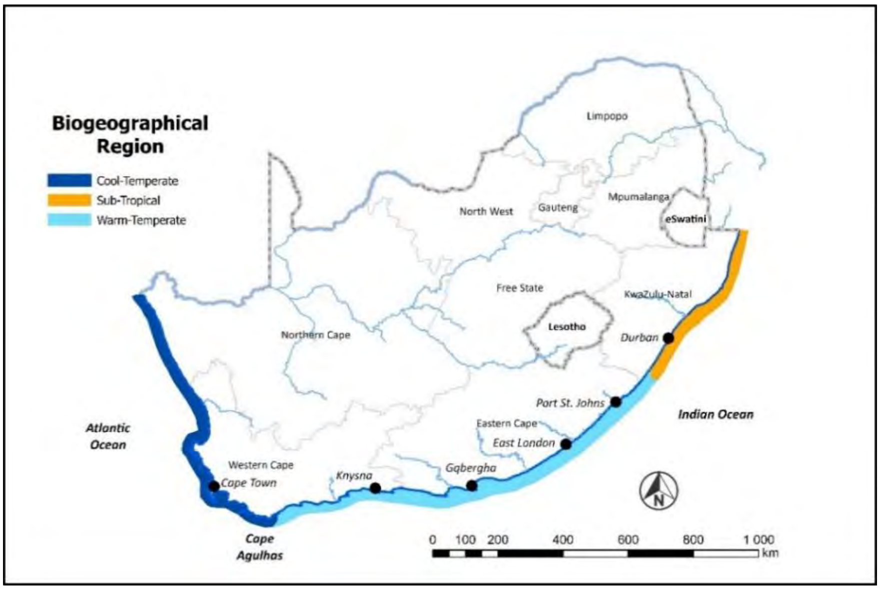


Figure 1.1: Biogeographic regions along the South African coastline (adapted from Potts et al. 2015)

South African estuaries fall within different physical categories, with large estuarine systems comprising of estuarine lakes, estuarine bays, estuarine lagoons, and predominantly open or large fluvially dominated systems, while smaller systems consisting of large and small temporarily closed estuaries, small fluvially-dominated or arid-predominantly closed estuaries and micro systems, which are either micro-estuary, micro-outlet or coastal waterfall systems (van Niekerk et al. 2020). These different estuarine types are categorised by differing tidal prisms, mixing processes, and salinity regimes (van Niekerk et al. 2020, Table 1.1).

Table 1.1: Whitfield (1992) typology of South African estuaries adapted from van Niekerk et al. (2020).

Type	Tidal prism	Mixing process	Average Salinity (Practical Salinity Scale)
Estuarine Bay	Large	Tidal	20 - 35
Permanently Open	Moderate	Tidal/riverine	10 - > 35
River Mouth	Small	Riverine	< 10
Estuarine Lake	Negligible	Wind	1 -> 35
Temporarily Open/Closed estuaries	Absent	Wind	1 ->35

1.1.1 Conservation in estuaries

Estuaries are under serious long-term threat of damage and destruction; lack of management and attention has meant that most estuarine habitats are subjected to increasing anthropogenic pressures (Turpie et al. 2003, Lamberth and Turpie 2003, Whitfield 2019). Estuaries are subject to indirect, upstream pressure, such as catchment utilization as well as direct anthropogenic stress from increasing numbers of people visiting or living in coastal zones (Lamberth and Turpie 2003). Contributing human pressures are freshwater abstraction, resource use, disturbance from boats, bait digging, eutrophication, pollution, and poorly placed and designed development (Adams et al. 2019, van Niekerk et al. 2019a). These effects are exacerbated by the ever-increasing impacts of climate change (James et al. 2019, van Niekerk et al. 2019b) and overexploitation (Cowley et al. 2013). Many estuary-associated fishery species are overexploited, and as a result, estuarine nursery habitats can create bottlenecks in their life history (Whitfield and Cowley 2013, Cowley et al. 2013). Excessive exploitation of estuarine fish can have significant consequences in estuarine and marine ecosystems, such as changes in the size of fish populations, estuarine biomass, age-sex-size and community profiles, and trophic structure (Pauly et al. 1998, Blaber 2000, Barange et al. 2004, Barletta and Costa 2009, Whitfield and Cowley 2010, van Niekerk et al. 2019a). Fish communities are, therefore, important indicators that can be used to assess estuarine health and assess management effectiveness. Understanding the role of estuaries, particularly the larger coastal lagoons, for coastal fishery species is becoming increasingly important and highlights the significance of monitoring fish communities for conservation and management.

In terms of conservation, estuarine habitats are difficult habitats to manage as fish utilising estuaries are placed under various degrees of pressure depending on what region of the estuary these fish species are using, be it marine, estuarine, or freshwater reaches of the system (Whitfield and Cowley 2010). The threats to estuarine fishes range from habitat degradation, disruption of essential ecological processes, hydrological manipulations, environmental pollution, overexploitation due to fishing activities, global effects, genetic contamination, and effects of introduced aquatic animals (Bruton 1995). Habitat degradation has been identified as one of the major threats and drivers of poor estuarine status currently in South Africa (Whitfield 2019). Although it is well-accepted that estuaries are usually more productive than adjacent marine waters, this high level of primary and secondary productivity is widely ignored when assessing the value, both economic and ecological, of these systems (Whitfield 2019). The fishery species targeted in South African estuaries are recreational fishery species, as the commercial fisheries species very seldom make use of estuaries, and those that generally do, do so at the very early juvenile life stage (Whitfield 2019). With an estimated recreational fishery participation of 1 327 633 people (Potts et al. 2022), the significant levels of fishing effort by this sector places huge amounts of pressure on the recreational fishery species utilising estuaries.

Effective planning of where priority conservation sites should be located and evaluating the various estuarine use options are essential components of developing national, regional, and local strategies for the conservation and sustainable use of biodiversity (Whitfield 2019). Many researchers worldwide have identified the importance of spatial data in developing conservation strategies (Mackelworth 2012, Selwood et al. 2019, Daigle et al. 2020, Colloca et al. 2015, Costa et al. 2016). Pressey et al. (2007) defined conservation planning as the process of locating, configuring, implementing, and maintaining areas that are managed to promote the persistence of biodiversity and other natural values, highlighting the inherently spatial nature of conservation. Colloca et al. (2015) stated that identifying nursery grounds and other essential fish habitats is a key requirement in developing spatial conservation planning aimed at reducing the adverse impact of fishing. To conserve an environment and its associated biodiversity, identifying biodiversity hotspots and essential habitats is crucial to prioritizing the most critical areas and allowing for a more targeted, efficient conservation plan.

Effective spatial planning has been identified as a critical aspect in the conservation of South African estuaries (Enviro-Fish Africa 2007, van Deventer et al. 2020) as developing spatial maps highlighting important biophysical features and anthropogenic features allow for better planning when developing conversation areas, earmarking areas for rehabilitation and planning future land use in and around estuaries (van Deventer et al. 2020). Large-scale spatial conservation research of South African estuaries has been undertaken by Turpie et al. (2002). They rated estuaries in terms of various factors and assigned conservation priority to those most in need of conservation. Although it would be ideal to ensure the high-quality functioning of all estuaries in South Africa, this is not practical, so short of this, Turpie et al. (2002)

identified the top priority estuaries in terms of conservation in the country and suggested prioritizing conservation efforts in these estuaries to ensure best overall functioning for coastal biodiversity. Turpie et al (2002) identified the Knysna Estuary as having the highest conservation importance of the 250 estuaries in South Africa. This classification was based on the system's weighted size, habitat, zonal type rarity, and biodiversity. While there was little information on the Knysna Estuary in 2002, this prioritisation has resulted in numerous ecological studies on the system (Appendix 1).

The Knysna Estuary is classified as an "estuarine bay" and due to its large size, acts as a small marine embayment, which leads to some species being observed in the system that would not normally appear in an estuarine environment. The Knysna Estuary is an open system that offers access to many marine fish species (South African National Parks 2017). Indeed, the Knysna Estuary is noted for having a high proportion of marine stragglers when compared with other estuaries in the area; due to the system having a strong marine influence, this leads to high species richness (James and Harrison 2008). The width of the Knysna Estuary mouth allows easy access for many marine species not usually associated with estuarine environments. For example, it has been noted that the estuary mouth does not act as a barrier for small pelagic fish species, which often attract predatory pelagic species not generally noted for their presence in estuaries (Grindley 1985). Bulpin (1978) stated that over 200 fish species had been recorded within the Knysna Estuary, although this list can no longer be found. Grindley (1985) constructed what is now generally considered to be the definitive list of common fish found in the Knysna Estuary, consisting of 60 different fish species from 38 families (Appendix 1). The Department of Forestry, Fisheries, and the Environment undertook biannual long term seine net sampling from 2007 to 2012, this sampling effort produced 54 different fish species from 32 families (Appendix 1). Work by Pollard et al. (2017) dealt with two different families, Mugilidae and Sparidae, examining the habitat usage differences between *Z. capensis* beds and bare sediment of four different Mugilidae species and five different Sparidae species (Appendix 1). The results from Pollard et al. (2017) showed that between the two families of interest, Mugilidae and Sparidae there was a difference in habitat usage with Sparidae species favouring the densely vegetated habitat and Mugilidae species favouring the sparsely vegetated habitat. Even within single species the habitat usage varied by length of fish. This shows there is a complicated relationship between fish and habitats within an estuarine system and determining which fish species are making use of specific habitats could play a vital role in protecting the biodiversity of fish species within these estuarine systems.

Previous research conducted by other authors in the Knysna Estuary (Appendix 1) has shown specific outcomes, largely focused on a small number of families or specific species and within limited spatial areas. Research conducted by James and Harrison (2008) was a more widespread sampling effort; however, it was temporally limited to 26 seine net hauls during the spring of 1994. The lastest widespread sampling in the Knysna Estuary was undergone by DFFE (2007-2012) making use widespread seine net sampling this work is however unpublished and made use of only a single sampling method. To date, there has also been no work published on demersal fish assemblages in the Knysna Estuary. The large marine influence in the system and observations of demersal marine species in the Knysna Estuary creates the need for a sampling method which properly targets these demersal fish species.

1.1.2 Conservation in the Knysna Estuary

It is well-accepted that the Knysna Estuary is vital as a nursery system for many fish species. This system is important in terms of conservation, with the presence of an estimated 42% of the country's estuarine biodiversity; the system also plays host to the endangered species *Zostera capensis*, *Hippocampus capensis,* and *Siphonaria compressa* (Claassens et al. 2020, Turpie et al. 2002). The economic value offered by the Knynsa Estuary’s nursery function is estimated at R167.6 million per annum with an estimated subsistence value of R786 500 (Turpie and Joubert 2005).

The Knysna Estuary was proclaimed a protected environment under the National Environmental Management Act 107 of 1998: Protected Areas Act (2009) and falls within the Garden Route National Park under the management of South African National Parks (SANParks). Regulations for the proper administration of the Knysna protected environment promulgated in 2009 (Government Gazette. No.R.1175) assigns power to SANParks to manage land use, access, and activities as well as monitor the use of biological resources. Zonation is one method used to achieve conservation and social objectives for the system (GRNP 2020).

Appropriate use zoning of the estuary serves to minimise conflicts between different users by separating potentially conflicting activities while ensuring that activities that do not negatively impact the estuary and its enjoyment by users can continue in appropriate areas. In terms of its conservation and management, the Knysna Estuary is currently divided into different "sensitivity zones" with a high, medium, or low rating (Figure 1.2), which determine how the estuary is managed. Higher sensitivity areas are areas of higher ecological importance. Within the current GRNP Management Plan (GRNP 2020), the Knysna Estuary was primarily zoned as low-intensity leisure, with a high-intensity leisure zone in the main channel (Figure 1.2). The current zonation identifies areas of high sensitivity, such as *Z. capensis* beds and saltmarshes, which are zoned as quiet (GRNP 2020). These sensitivity ratings are based on habitat type, which is seen as an indicator of conservation importance, and not, however, based on any fish assemblage data.

Special management areas have been implemented in the Knysna Estuary based on habitat sensitivity; restricted regions in the estuary include bait exclusion, no motorized vessels, no wake channel, and idle speed intertidal areas (GRNP 2020) (Figure 1.3). SANParks has also enforced special management areas within the Knysna Estuary which have specific restrictions in terms of activities (GRNP 2020) (Figure 1.3). The bait exclusion region was demarcated to protect bait species as well as other fauna and flora on intertidal mudflats and sandbanks (GRNP 2020). Regions where motorised vessels have no access are designated due to environmental sensitivity of the area, this is generally applied to shallow areas where motorised vessels can cause erosion and damage to sensitive intertidal areas, such as mudflats, *Z. capensis* beds and salt marsh vegetation (GRNP 2020). The no wake channels generally apply to shallow channels adjacent to sensitive estuarine habitats or areas where there are potential safety issues due to moored vessels or infrastructure (GRNP 2020). Idle speed zones are situated over environmentally sensitive areas which are only accessible at high tide and vessels are required to reduce speed in order to not create wake which can damage the environment (GRNP 2020).

Although habitat type is often a good indicator of assemblages, estuaries form very complicated interlinking habitats, and setting up sensitivity zones based specifically on fish distribution is more effective at conserving fish than predicting distributions based on habitat type. In order to determine whether these sensitivity zones are appropriate for conserving fish species in the system, it is important to determine how different fish species are distributed throughout the system, which is research that has not currently been undertaken in the Knysna Estuary. Identifying and safeguarding juvenile hotspots is crucial in protecting species as a whole and allowing them the best opportunity for continued survival. In a system such as the Knysna Estuary, which is under heavy anthropogenic stress, identifying juvenile hotspots is essential in protecting and managing the exploitation of these fishes.

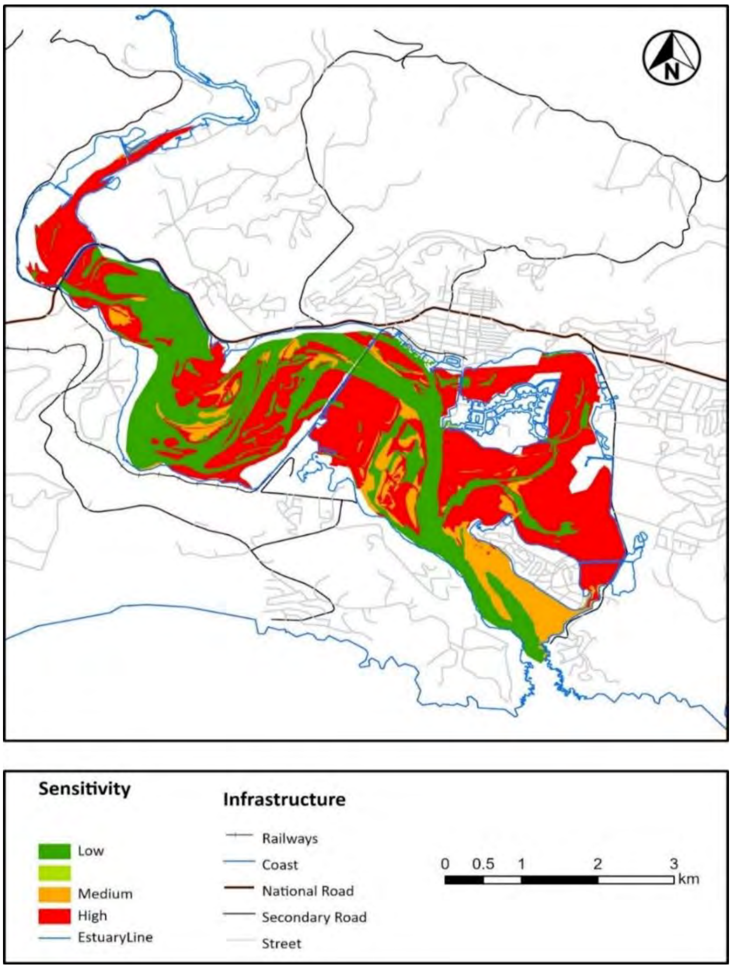


Figure 1.2: Ecological sensitivity zones within the Knysna Estuary areas are given low, medium or high sensitivity value as set out by SANParks in order to best manage the system (Shapefile provided by SANParks, GRNP 2020)

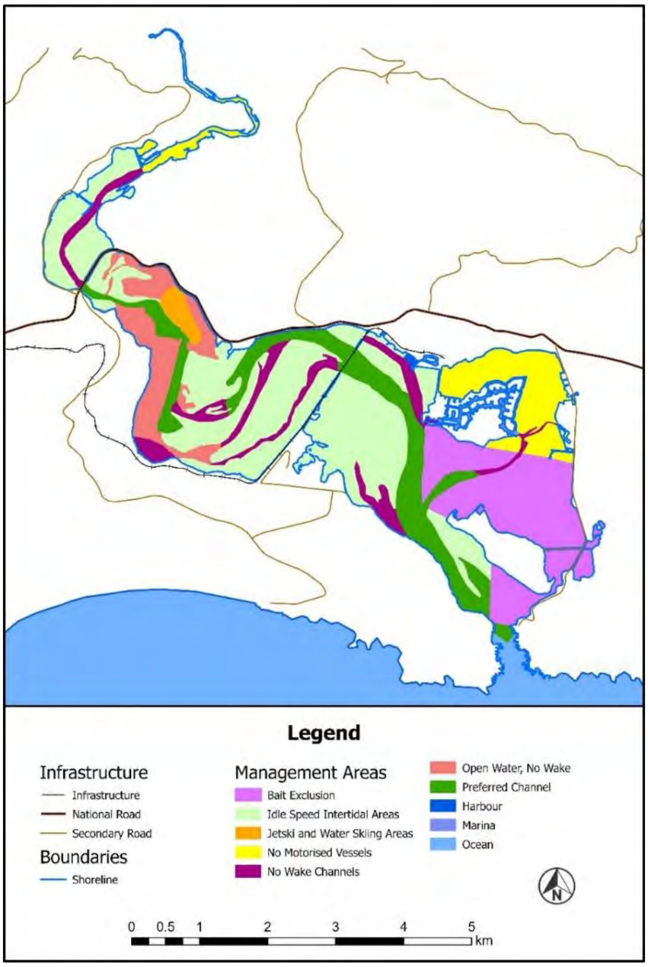


Figure 1.3: Current estuarine special management areas in place in the Knysna Estuary (adapted from GRNP 2020)

1.2 Motivation for study

Although the Knysna Estuary has been well-studied due to its importance in terms of conservation, there is very little recent research on overall fish assemblages in the system, with the demersal fish species being largely ignored. In an estuarine system with marine influence as great as the Knysna Estuary, demersal marine species and previously ignored demersal estuarine species could play an important part in the overall fish community in the system. By developing spatial mapping data for fish distribution in the Knysna Estuary, it is possible to provide conservation guidelines for protecting the fish resource within the Knysna Estuary.

1.3 Research aims and objectives

The main aim of this study is to assess the spatial distribution of fish assemblages within the Knysna Estuary. In order to achieve this aim, two different sampling methods were used, one that best samples the littoral fish populations and one that samples the previously ignored demersal fish populations within this system. Determining the spatial distribution of fish within the system will allow for a spatially guided approach to conserving the fish species within the system.

The specific objectives of this study were to:

i. Identify what fish species are present in the Knysna Estuary.
ii. Use spatial mapping to determine what regions of the estuary were favoured by different estuarine fish guilds.
iii. Determine the spatial distribution of life stages of key fish species throughout the estuary.
iv. Identify key juvenile hotspot habitats of key species in the estuary.

1.4 Thesis structure

This thesis is divided into five chapters, including general introduction (Chapter 1), study site and general materials and methods (Chapter 2). Seine netting was used as a sampling method to provide a spatial assessment of the littoral fish distribution (Chapter 3) whilst beam trawls were used to target the demersal fish assemblages (Chapter 4). Chapter 5 discusses the findings from the study in the context of spatial conservation planning, as well as provides guidelines for implementing the results of the study in order to best spatially manage fish resources in the Knysna Estuary.

CHAPTER TWO

STUDY SITE AND GENERAL MATERIALS AND METHODS

2.1 Introduction

The Knysna Estuary is located in the town of Knysna in the Western Cape of South Africa (34°4'35" S 23°3'35" E). It is classified as an "estuarine bay," being one of two naturally occurring estuarine bays in South Africa: Knysna Estuary and Durban Bay (van Niekerk et al. 2020). However, Durban Bay is partly an artificial system due to dredging activities (Whitfield 2019). The Knysna Estuary is permanently open, and there are no impoundments on the inflowing rivers. The Knysna Estuary is the only estuary of its kind in the warm temperate bioregion of South Africa, which extends from the Mdumbi Estuary, along the east coast to Cape Agulhas at the western edge of the south coast (Harrison 2002) (Chapter 1, Figure 1.1).

The Knysna Estuary is the largest estuary along the southern coast of South Africa (Marker 2003), covering 1 633ha (Duvenhage 1983). The estuary's main channel runs for around 19 km, of which 17 km is tidal with a maximum width of 2 km (South Africa National Parks 2017, James and Harrison 2008). The Knysna River, which flows into the estuary, has a length of 64 km (Day et al. 1952) with a catchment area of between 315 and 337 km^2 (Grindley 1985). The mouth to the Knysna Estuary is characterised by the "heads," with cliffs on either side that create a narrow and deep channel approximately 200 m wide (James and Harrison 2008) and a depth of 15 m (South African National Parks 2017). Differing maximum depths have been reported for the estuary that range from 12 m (Krige 1927) to 16 m (Day 1981). Research from the 1970s suggests that there could be areas of the estuary deeper than 16 meters below sea level (South African National Parks 2017). The estuary's main channel follows the broad turns of the river, getting progressively shallower upstream to a depth of 2 m at the maximum tidal reach, around 17 km upstream of the mouth (South African National Parks 2017). An abundance of inter- and subtidal sandbanks occur along the main estuary channel. There are two islands present in the lower reach of the estuary, namely Leisure Island and Thesen Island, both of which are connected to the mainland via bridges (Figure 2.1).

Three distinct zones have been identified in the system as the sampling regions for this study (Largier et al. 2000). This includes the 'marine bay,' which is closest to the mouth and is the area of this estuary that makes the system unique in the context of warm-temperate South African estuaries (Figure 2.1). This region of the estuary flushes with every tidal cycle and shows similar physiochemical characteristics to the ocean adjacent to the estuary (Switzer 2003). The 'lagoon' section extends inland from the bay (Figure 2.2) and is a region generally associated with higher water temperature due to low tidal exchange and little freshwater influence (Largier et al. 2000). The upper tidal reaches of the estuary form the estuarine region of the system, which is characterised by lower salinity (Largier et al. 2000). For the purpose of this study, the estuary can be divided into two different regions, the ‘lower estuary’ and the ‘upper estuary’ (Figure 2.1).

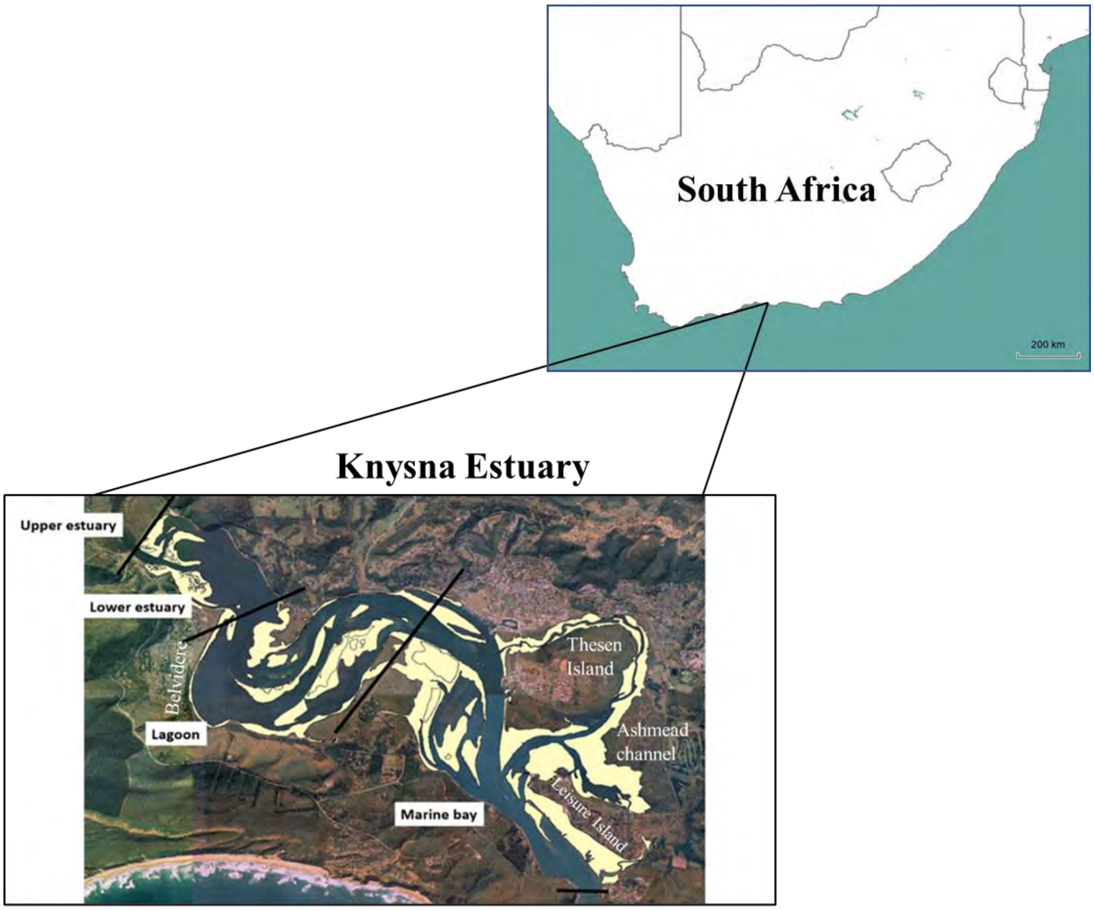


Figure 2.1: Map of South Africa is regional inset of the Knysna Estuary illustrating four distinct estuarine regions/zones, "marine bay," "lagoon," and the estuary broken up into the "lower estuary" and "upper estuary" (adapted from Largier et al. 2000). Key regions of the estuary highlighted, "Belvidere", "Thesen Island", "Ashmead Channel" and "Leisure Island". Light shaded area: Intertidal sand and mudflats

Sampling was undertaken in June 2021, November 2021 and March 2022 at 30 seine net (littoral) sites and 25 (demersal/channel) beam trawl sites (Figure 2.2). Sampling sites were selected and distributed in order to best cover the entire area of the Knysna Estuary and the widest variety of habitats possible.

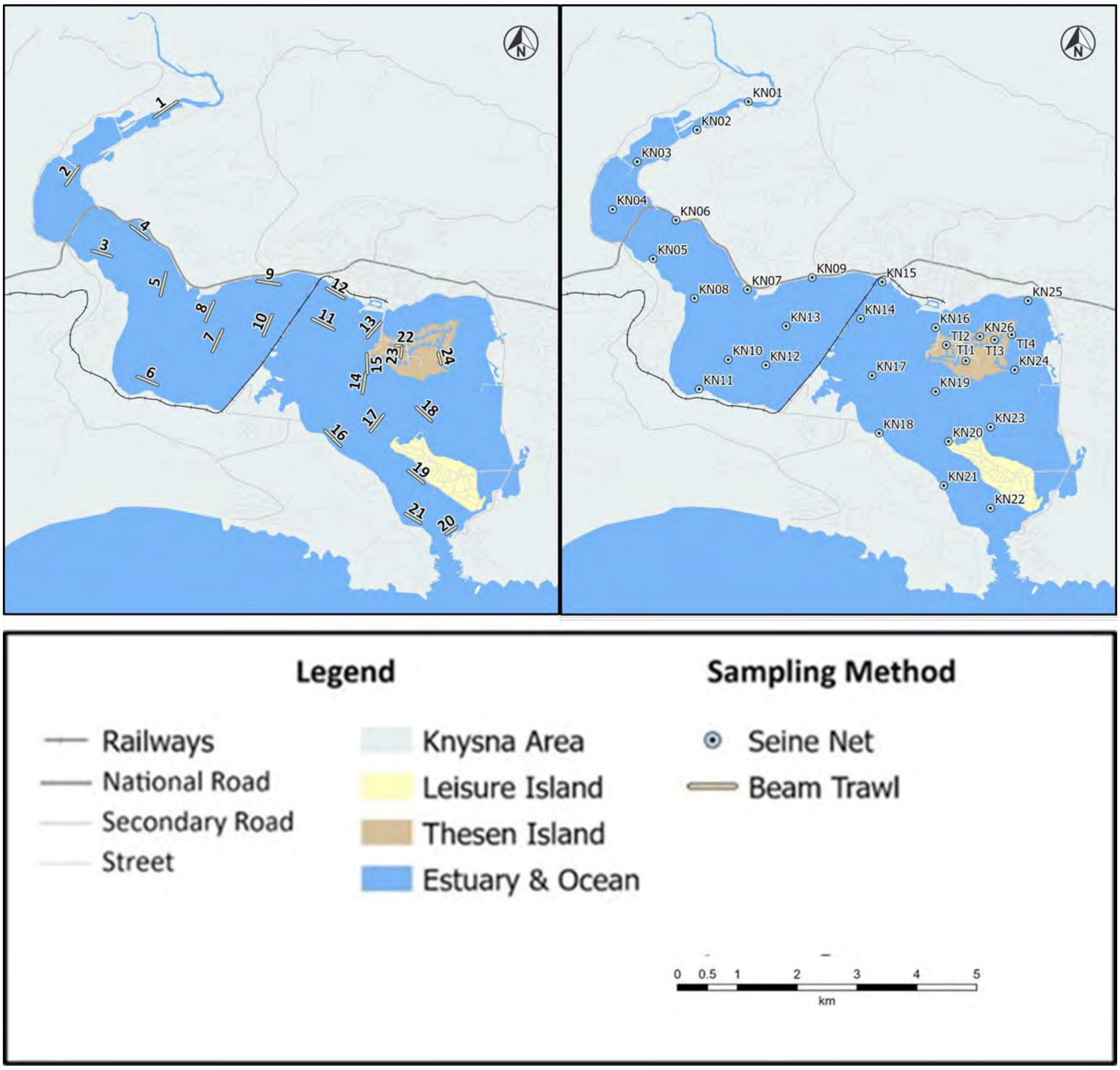


Figure 2.2: Map of the Knysna Estuary highlighting various aspects of the study site, including all sample sites for both sampling methods. Seine net sites labelled S01-S30, beam trawl sites labelled B01-B025.

2.2 Physiochemical Parameters

Various *in situ* physicochemical parameters (temperature (°C), salinity, dissolved oxygen (mg.l^{-1}), and turbidity (NTU)) were measured in surface-to-bottom water profiles at each sampling site using a Hanna instruments ® HI98196 Multiparameter meter.

2.2.1 Temperature

Surface temperatures measured varied greatly depending on the time of year when sampling was undertaken; the first sampling trip was a winter seine net trip (14 June – 18 June 2021) and showed the lowest average temperature of 16.86 °C (± 0.69 °C). The other sampling trips were made up of summer efforts, with the first beam trawl sampling trip (16 November – 17 November 2021) recording an average temperature of 22.01 °C (± 1.20°C), the second seine net sampling trip (21 February – 23 February 2022) recording an average temperature of 20.56 °C (± 2.08 °C) and the second beam trawl sampling trip (3 March – 4 March 2022) recording an average temperature of 20.19 °C (± 0.90 °C). Especially notable is the significant variation in temperatures for the second seine net trip, with a minimum temperature of 16.42 °C and a maximum temperature of 25.41 °C. This wide variability in temperatures is commonly associated with summer upwelling patterns. Coastal upwelling of waters adjacent to the Knysna Estuary can cause a sudden drop in water temperatures to 11.5°C in the mouth region of this system (Day 1981). Indeed, summer upwelling is a regular feature affecting this estuary following persistent easterly winds; Schumann (2000) recorded a temperature decline of over 13°C in two hours. These lower temperatures during this sampling effort were also more notable in the sampling sites in the marine bay region of the estuary. Estuaries in South Africa are known to provide thermal refuge for species during coastal upwelling events (Hanekom et al. 1989). During rapid declines in coastal water temperatures, estuarine water temperatures tend to remain more constant, thus buffering the upwelling effect. In a system such as the Knysna Estuary, where adjacent coastal upwelling is a relatively common phenomenon, many marine fish species are thought to move into the system during upwelling events, with anglers targeting marine species such as *Spardon durbanesis* when the adjacent ocean temperature drops due to upwelling (K. Smith pers.comm). During a summer sampling effort, a smooth hammerhead shark (*Sphyrna zygaena)*, a marine-associated shark species, was noted swimming on the surface just above the mouth of the system.

2.2.2 Salinity

The salinity measured during the sampling effort ranged from 28 to 39, with sites in the upper estuary region with an average salinity below 30. Although a general decline in salinity was recorded further up the system due to the significant tidal exchange in the system, much of the lagoon region of the system showed salinity values similar to that of ocean water (37). Average salinity values of up to 36 were recorded as high as B03, which falls in the lower estuary region of the system. Although the salinity values recorded in this study vary slightly to what has been previously recorded by James and Harrison (2008) which was undergone in 1994 (range: 23 to 35), the salinity values recorded in both studies fall within the polyhaline (18-30 PSU) and euhaline (>30PSU) range of the Venice Salinity Scale. It is also difficult to compare salinity values to James and Harrison (2008) as no precise site information was provided in this study, just that 6 sites from the lower reaches to the upper reaches were used.

2.2.3 Turbidity

The Knysna Estuary is generally associated with very low turbidity (Adams 2016), with the large tidal exchange leading to clear water dominating much of the system. Turbidity readings recorded during the sampling trips ranged from 0 to 21.1 NTU, with 50% of measurements recording 0 NTU. These values show a similar range to what was recorded in 1994 by James and Harrison (2008), which show turbidity values ranging between 2 and 18 NTU, comparing the results is however difficult without specific site information for the work by James and Harrison (2008). The Knysna River flows through large areas of indigenous forest known as the Knysna-Amatole forests (Koen and Crowe 1987). The tannin from the upstream forests results in darker water with a reddish hue. The high levels of suspensoids in the inflowing tannin-stained water results in the system's upper reaches having higher turbidity levels than the lower reaches (Figure 2.3).

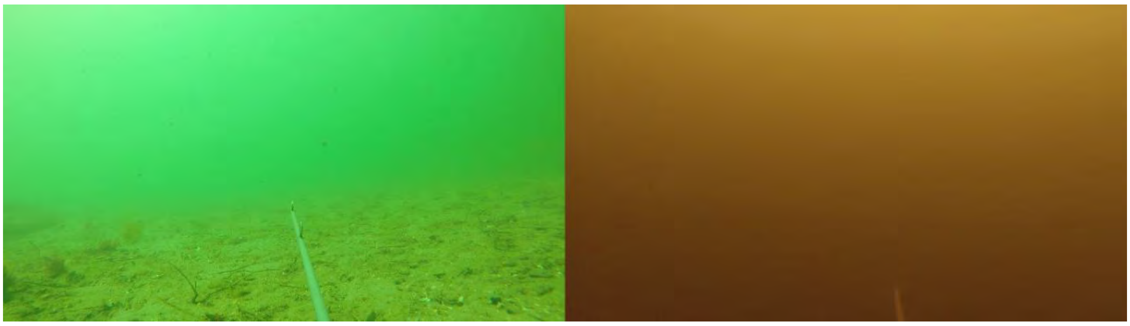


Figure 2.3: Comparison of turbidity between clear water marine dominated marine bay sites and a higher turbidity upper estuary site within the Knysna Estuary (images obtained from camera footage)

2.2.4 Dissolved oxygen

Dissolved oxygen measured during the sampling trips showed significant variability, with sites in the upper regions of the system generally recording lower dissolved oxygen. Dissolved oxygen readings < 50% were measured during beam trawl sampling in the upper estuary region, whereas most of the sites in the lagoon and marine bay regions measured values of > 90% with a high at site S19 on the outer edge of Thesen Island in the marine bay region. The constant mixing of ocean water due to the significant tidal exchange in the lower region of the system leads to the high dissolved oxygen content in the marine bay and lagoon. The shallower, less tidally affected sites in the estuary regions of this system, where less mixing of water occurs, are associated with lower dissolved oxygen levels. Dissolved oxygen readings done in 1994 by James and Harrison (2008) differ from the readings for this study with a much narrower value range of between 69.6% and 80.85% although without specific site information for these values it is difficult to compare results.

Table 2.1: Knysna upper estuary region sites average (± Sd) water quality parameters over two sampling periods recorded at each site, including temperature (°C), salinity, pH, turbidity (NTU) and dissolved oxygen (%). Standard deviation for each site and parameter included.

Sample#	**Temperature (°C)**	**Salinity (ppt)**	**pH**	**Turbidity (NTU)**	**Dissolved Oxygen (%)**
S01	18.6 (±4.0)	25.5 (±0.7)	7.0 (±0.6)	0.3 (±0.5)	87.9 (±18.0)
S02	19.1 (±3.4)	25.5 (±2.1)	7.0 (±0.2)	0.5 (±0.7)	80.6 (±6.8)
S03	19.0 (±2.9)	25.5 (±0.7)	7.2 (±0.5)	2.8 (±2.2)	77.8 (±7.4)
S04	19.8 (±3.0)	29.0 (±1.4)	7.4 (±0.5)	1.0 (±1.5)	91.4 (±5.4)
B01	23.2 (±1.7)	29.0 (±1.4)	7.5 (±>0.1)	0.3 (±0.3)	44.1 (±2.8)
B02	22.5 (±1.9)	30.5 (±2.1)	7.7 (±0.2)	2.8 (±3.9)	45.3 (±12.7)

Table 2.2: Knysna lower estuary region sites average (± Sd) water quality parameters over two sampling periods recorded at each site, including temperature (°C), salinity, pH, turbidity (NTU) and dissolved oxygen (%). Standard deviation for each site and parameter included.

Sample#	**Temperature (°C)**	**Salinity (ppt)**	**pH**	**Turbidity (NTU)**	**Dissolved Oxygen (%)**
S05	17.3 (±2.9)	29.5 (±0.7)	7.5 (±0.3)	0.2 (±0.3)	89.4 (±0.8)
S06	19.8 (±3.7)	28.5 (±0.7)	7.5 (±0.5)	10.4 (±6.4)	94.8 (±19.7)
S08	19.0 (±2.9)	25.5 (±0.7)	7.2 (±0.5)	2.8 (±2.2)	77.8 (±7.4)
B03	22.2 (±1.7)	36.0 (±1.4)	8.0 (±0.2)	0.1 (±0.1)	67.8 (±19.2)
B04	22.1 (±1.8)	35.0 (±1.4)	8.0 (±0.1)	0.7 (±0.7)	68.3 (±13.2)
B05	22.1 (±1.6)	35.5 (±0.7)	8.1 (±0.2)	4.1 (±3.7)	69.0 (±12.5)

Table 2.3: Knysna lagoon region sites average (± Sd) water quality parameters over two sampling periods recorded at each site, including temperature (°C), salinity, pH, turbidity (NTU) and dissolved oxygen (%). Standard deviation for each site and parameter included**.**

Sample#	**Temperature (°C)**	**Salinity (ppt)**	**pH**	**Turbidity (NTU)**	**Dissolved Oxygen (%)**
S07	17.3 (±2.9)	29.5 (±0.7)	7.5 (±0.3)	0.2 (±0.3)	89.4 (±0.8)
S09	19.4 (±3.9)	31.5 (±2.1)	7.8 (±0.6)	3.6 (±5.0)	95.2 (±4.0)
S10	19.0 (±3.2)	30.5 (±0.7)	7.6 (±0.5)	7.9 (±6.4)	102.3 (±7.6)
S11	19.7 (±4.7)	31.0 (±1.4)	8.2 (±1.1)	5.1 (±6.8)	104.6 (±6.4)
S12	19.3 (±3.0)	31.0 (±>0.1)	7.9 (±0.6)	0.0 (±0.0)	96.8 (±2.0)
S13	19.4 (±3.4)	29.0 (±1.4)	7.9 (±0.6)	0.5 (±0.6)	110.4 (±4.7)
B06	22.1 (±2.2)	35.8 (±0.2)	8.4 (±0.5)	1.4 (±1.9)	89.5 (±24.3)
B07	21.6 (±0.9)	36.3 (±1.8)	8.2 (±>0.1)	2.2 (±3.1)	97.2 (±14.5)
B08	21.1 (±0.3)	37.9 (±0.2)	8.2 (±0.2)	1.2 (±1.8)	86.5 (±23.8)
B09	21.2 (±0.1)	36.1 (±1.6)	8.3 (±0.1)	1.7 (±2.4)	91.0 (±23.8)
B10	21.2 (±>0.1)	37.0 (±>0.1)	8.3 (±0.2)	2.5 (±3.5)	103.1 (±13.6)

Table 2.4: Knysna marine bay region sites average (± Sd) water quality parameters over two sampling periods recorded at each site, including temperature (°C), salinity, pH, turbidity (NTU) and dissolved oxygen (%). Standard deviation for each site and parameter included.

Sample#	**Temperature (°C)**	**Salinity (ppt)**	**pH**	**Turbidity (NTU)**	**Dissolved Oxygen (%)**
S14	18.9 (±2.8)	31.0 (±1.4)	7.9 (±0.7)	1.4 (±1.9)	95.5 (±0.3)
S15	18.7 (±2.7)	33.0 (±>0.1)	7.7 (±0.5)	0.4 (±0.6)	108.7 (±28.9)
S16	18.4 (±3.1)	34.5 (±0.7)	7.7 (±0.5)	0.7 (±0.5)	102.5 (±30.4)
S17	18.3 (±1.3)	35.5 (±0.7)	8.0 (±0.5)	0.1 (±0.1)	106.8 (±18.5)
S18	18.1 (±1.3)	34.0 (±1.4)	7.9 (±0.7)	1.4 (±1.9)	101.5 (±0.3)
S19	18.1 (±1.6)	35.5 (±2.1)	7.6 (±>0.1)	0.0 (±0.0)	109.5 (±21.6)
S20	19.0 (±1.5)	35.0 (±1.4)	7.8 (±0.5)	1.4 (±2.0)	112.3 (±2.0)
S21	17.2 (±0.1)	36.0 (±1.4)	7.9 (±0.6)	0.9 (±1.3)	94.2 (±2.5)
S22	19.0 (±1.1)	37.0 (±1.4)	7.9 (±0.6)	0.4 (±0.6)	93.4 (±12.7)
S23	20.5 (±4.2)	35.0 (±>0.1)	8.0 (±0.7)	10.6 (±14.9)	111.0 (±2.0)
S24	18.6 (±2.7)	30.0 (±2.8)	7.7 (±0.5)	2.8 (±3.9)	102.9 (±20.7)

S25	17.7 (±2.0)	33.0 (±1.4)	7.5 (±0.4)	9.9 (±13.8)	81.3 (±5.7)
S26	17.4 (±0.5)	31.0 (±4.2)	7.7 (±0.5)	4.4 (±6.3)	107.6 (±8.7)
S27	16.5 (±0.1)	33.0 (±1.4)	7.8 (±0.5)	0.6 (±>0.1)	103.4 (±14.4)
S28	16.9 (±>0.1)	33.0 (±1.4)	7.7 (±0.5)	2.3 (±>0.1)	103.1 (±14.9)
S29	18.8 (±1.3)	33.5 (±2.1)	7.7 (±0.4)	1.4 (±0.6)	104.1 (±5.0)
S30	18.3 (±1.5)	35.0 (±2.8)	7.7 (±0.4)	3.9 (±2.1)	107.8 (±9.1)
B11	21.0 (±0.4)	36.6 (±0.8)	8.3 (±0.2)	4.0 (±5.6)	99.8 (±20.3)
B12	20.9 (±0.5)	36.0 (±1.4)	8.3 (±0.2)	0.0 (±0.0)	97.8 (±20.1)
B13	20.6 (±2.8)	37.8 (±1.7)	8.4 (±0.2)	0.0 (±0.0)	106.7 (±25.5)
B14	19.9 (±2.0)	35.9 (±1.2)	8.4 (±>0.1)	0.0 (±0.0)	94.2 (±2.0)
B15	20.3 (±1.5)	37.4 (±0.9)	8.5 (±>0.1)	0.0 (±0.0)	107.8 (±17.2)
B16	19.9 (±1.0)	37.5 (±0.8)	8.4 (±>0.1)	0.0 (±0.0)	101.7 (±22.8)
B17	20.6 (±1.0)	35.2 (±1.7)	8.2 (±0.2)	0.0 (±0.0)	59.4 (±8.2)
B18	20.8 (±1.3)	35.4 (±0.5)	8.2 (±0.2)	0.0 (±0.0)	60.1 (±7.8)
B19	20.6 (±1.6)	36.6 (±0.5)	8.4 (±>0.1)	0.0 (±0.0)	104.3 (±13.9)
B20	20.1 (±0.7)	36.8 (±0.2)	8.4 (±>0.1)	0.0 (±0.0)	110.0 (±9.5)
B21	19.2 (±0.3)	37.5 (±0.7)	8.4 (±>0.1)	0.0 (±0.0)	108.0 (±7.3)
B22	21.0 (±1.9)	35.4 (±0.6)	8.3 (±0.1)	0.6 (±0.8)	90.3 (±11.2)
B23	21.0 (±1.9)	35.4 (±0.6)	8.3 (±0.1)	0.6 (±0.8)	88.8 (±13.3)
B24	21.4 (±2.0)	35.5 (±0.7)	8.1 (±>0.1)	0.0 (±0.0)	89.8 (±17.6)
B25	21.3 (±1.7)	35.5 (±0.7)	8.2 (±>0.1)	0.0 (±0.0)	89.6 (±19.7)

2.3 Knysna Estuary Habitat Types

The Knysna Estuary offers a wide variety of habitats with numerous shallow water banks as well as deep water in the main channel of the estuary (Figure 2.4). The dominant littoral vegetation types are seagrass *Zostera capensis* (Figure 2.5) and macroglae *Codium tenue* (Figure 2.6) and *Caulerpa filiformis* (Figure 2.7). The Knysna Estuary is a vital estuary in the context of South African estuaries due to the high area of endangered *Z. capensis* habitat found in the system (Adams 2016). The Knysna Estuary is home to 49% of the total South African *Z. capensis* beds (Grindley 1985, Russell et al. 2012), making a total area of 238 ha in this system (Schmidt 2013). These *Z. capensis* beds are regarded as critical estuarine habitats due to the vital functions they provide in supporting populations of many fish species, including important economic and recreational fish species (Pollard et al. 2018). The *Z. capensis* beds are widely distributed throughout the system, found in the system's marine bay, lagoon, and lower estuary regions (Figure 2.4). A large area of the Knysna Estuary comprises estuarine open water habitats (Figure 2.8, Figure 2.9) on various substrates such as sand banks in the lower reaches of the estuary, mudbank in the upper reaches of the estuary and areas with rocky bottoms.

Codium tenue is a macroalgae found in many different habitats but is especially associated with areas sheltered from waves as it is not attached to the bottom (Bulleri and Airoldi 2005). In the Knysna Estuary, the Thesen Island canals offer this wave-sheltered area leading to a large amount of *C. tenue* settling in these man-made canals (Figure 2.4). This accumulation of *C. tenue* in the Thesen island canals has been identified as an essential habitat for the critically endangered Knysna seahorse, *Hippocampus capensis*, and has been highlighted as an essential habitat to protect in order to conserve this flagship species for the Knysna Estuary (de Villers 2019). *Caulerpa filiformis* tends to be more abundant in the lagoon region (Teske et al. 2007), these habitats are present throughout the upper marine bay region to the lower estuary region, with a high prevalence of this habitat type in the middle reach of the system (Figure 2.4). *C. filiformis* is a habitat type which has received attention due to its usage by *H. capensis* (Teske 2007

The upper intertidal salt marshes have been shown to be an important fish nursery and feeding areas with fish communities in these salt marshes dominated by euryhaline marine taxa (Le

Quesne 2000). Although the Knysna Estuary salt marshes make up a relatively small area of the system (15%), they appear to play a relatively important role in the ecology of the system, supporting a large number of juvenile euryhaline marine species (Le Quesne 2000).

In order to best describe the habitats at sampling sites GoPro® cameras were dropped at each site and left for 60 minutes. The footage from these cameras was analysed to best view the subtidal vegetation and sediment type for these sample sites.

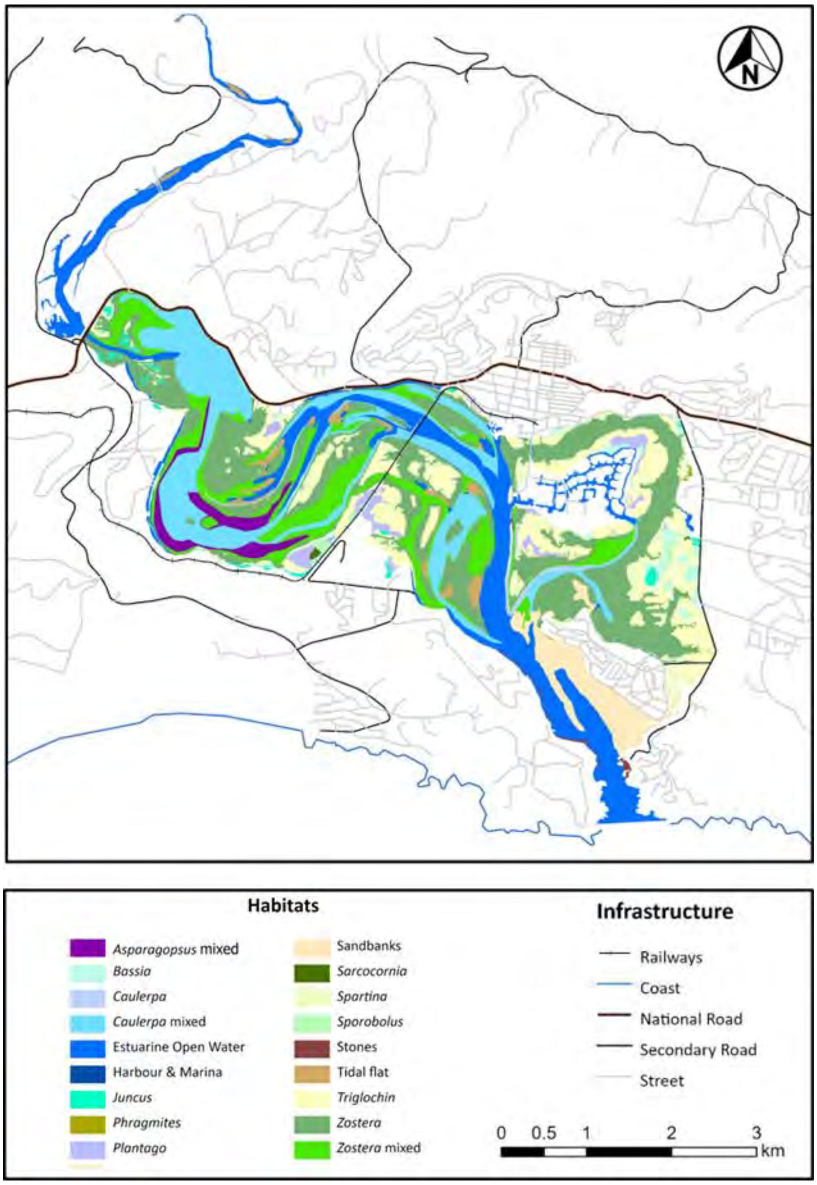


Figure 2.4: Map of the Knysna Estuary with different estuarine habitats (GIS shapefiles provided by SANParks (Wasserman et al. 2020)) Map generated May 2022

Figure 2.5: Camera footage illustrating *Zostera capensis* vegetation present at a site in the lower estuary region of the Knysna Estuary.

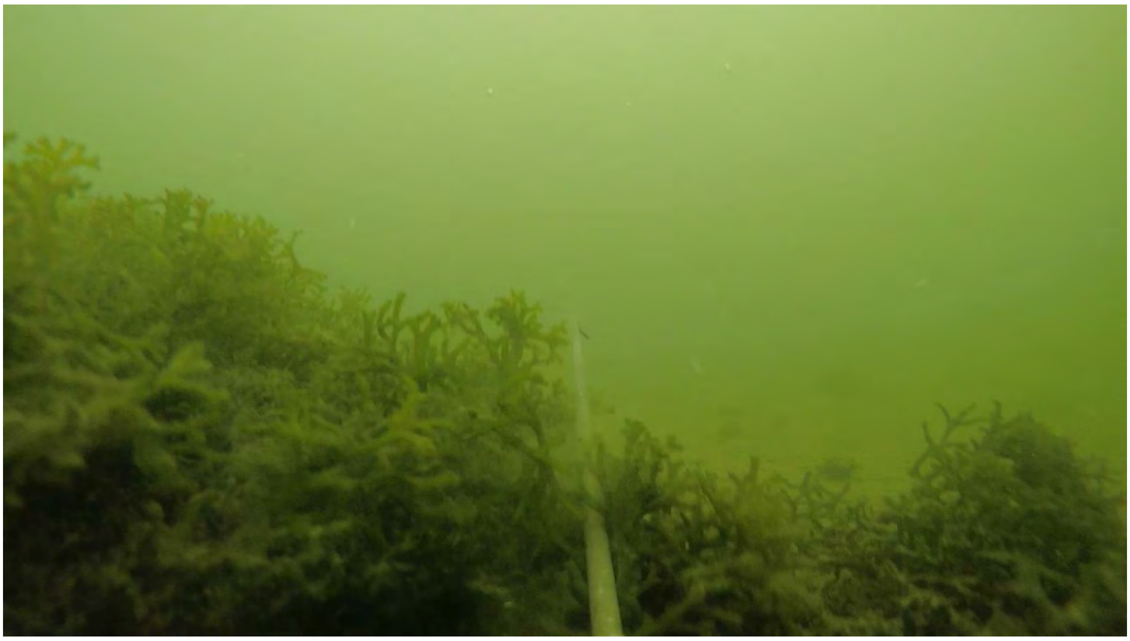


Figure 2.6: Camera footage illustrating *Codium tenue* macroalgal beds present in a site in the Thesen Island canals in the marine bay region of the Knysna Estuary.

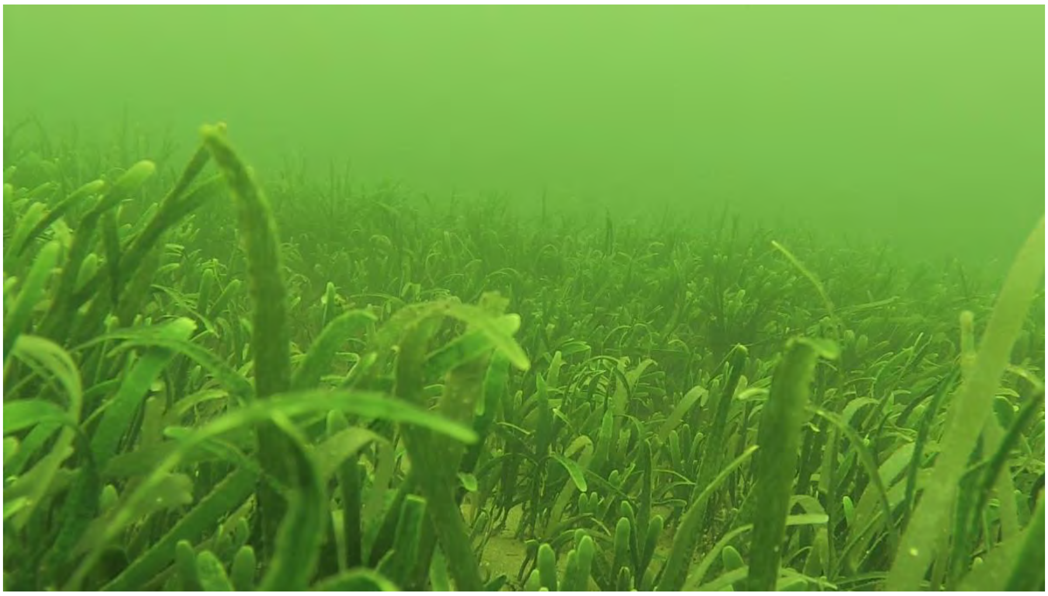


Figure 2.7: Camera footage illustrating *Caulerpa filiformis* macroalgal beds present in a site below the railway bridge in the marine bay region of the Knysna Estuary.

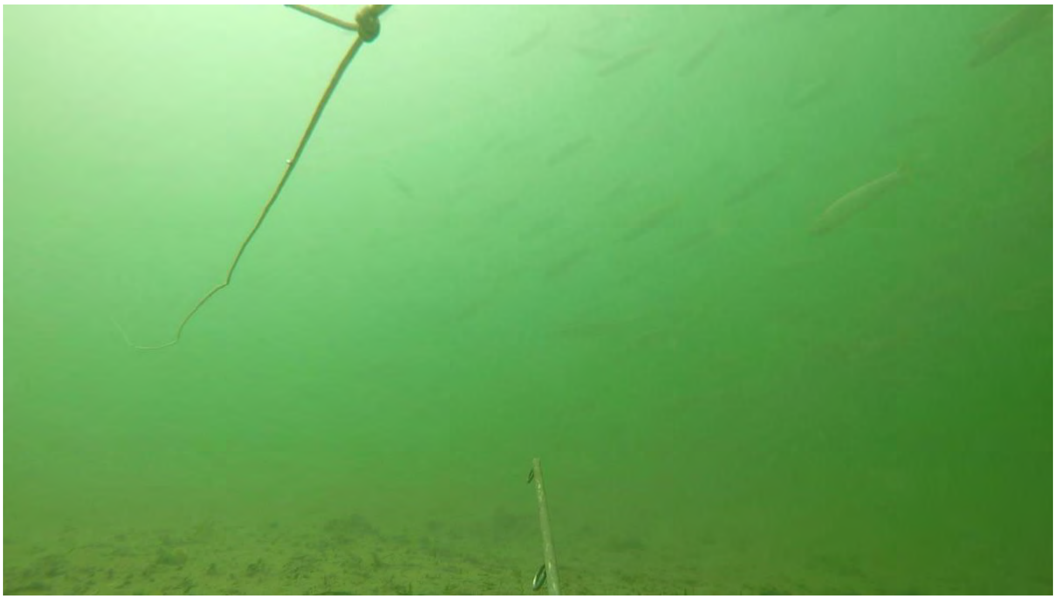


Figure 2.8: Camera footage illustrating a school of Mugilidae swimming above camera at a deep open water site in the estuary mouth in the marine bay region of the Knysna Estuary.

Figure 2.9: Camera footage illustrating open estuarine water sand bank habitat taken at the site nearest to the mouth in the marine bay region of the Knysna Estuary.

2.4 Research Approach and Sampling Sites

2.4.1 Fish Handling

All fish caught were placed in multiple buckets and holding nets while being sorted. Fish were handled carefully using wet work surfaces and wet hands to minimise stress and post-release fatalities. Where possible, fish were identified to a species level, total length measured (mm), and counted. For cryptic species and families, and where juveniles were difficult to identify, fish were identified to the lowest taxonomic level possible.

Fish that could not be identified to species level in the field were anesthetised and euthanised followed SANParks Standard Operating Procedures for the Capture, Scientific Manipulation, Anesthesia, and Euthanasia of Fish in South African National Parks (Petersen et al. 2020). Fish were anesthetised using 40 mg/l Clove oil, after which they were euthanised by ice immersion. These specimens are to be identified using DNA barcoding for future publications from the output of this study.

2.4.2 Estuarine guild classification

For data analysis in Chapter 3 and Chapter 4, all of the fish species sampled were separated into estuarine functional guilds (Potter et al. 2015) as applied by Whitfield (2019) for fishes occurring in South African estuaries (Table 2.5). There are 11 different estuarine-associated guilds, although they are often not all represented in an estuarine system. The marine (M) guild are species not recorded in estuaries, the marine-associated estuarine guilds, marine stragglers (MS), are species occasionally recorded in low numbers in estuaries, marine estuarine-opportunists (MEO) are species that enter estuaries in large numbers during a specific life stage, and marine estuarine-dependents (MED) are species reliant on estuaries for their survival during juvenile life stages (Potter et al. 2015). Estuarine species that can complete their entire life cycles within estuaries are either solely estuarine (SE) species, estuarine and marine (E&M) species, or estuarine and freshwater species (E&F) (Potter et al. 2015). Estuary migrant (EM) are species whose larvae are flushed out to sea and juveniles that survive will then return to the estuarine system as small juveniles (Potter et al. 2015). Catadromous species (Ca) are species that spend majority of their life cycle in freshwater while migrating to marine habitats to reproduce (Potter et al. 2015). Freshwater species (Freshwater stragglers (FS) and freshwater-estuarine opportunists (FEO)) tend to make up a low proportion of both the number and biodiversity of fish within estuarine habitats, and they tend to be restricted to the low salinity upper reaches of estuaries (Potter et al. 2015).

Table 2.5: Estuarine functional guild classification of fish utilizing estuaries (as defined by Potter et al. 2015)

Description of Category	
marine	M
marine straggler	MS
marine-estuarine opportunist	MEO
marine-estuarine dependent	MED
solely estuarine	SE
marine & estuarine	M&E
estuarine & freshwater	E&F
estuarine migrant	EM
catadromous	Ca
freshwater straggler	FS
freshwater-estuarine opportunist	FEO

2.4.3 Fish life stages

For the life stage distribution analysis of key fish species, the length data collected from sampled fish was used. The generally accepted fish life stage categories can be divided into five life stages using Kendall et al. (1984) classification scheme (Table 2.3). These include Postflexion larvae and Transformation stage, which are specimens less developed than Settlement stage individuals, Young-of-the-Year juveniles, which fall between the Settlement stage and Juvenile specimens, late juveniles, and adult stage. However, identifying fish specimens to the postflexion and transformation stage level requires the specimens to be kept, and for a sampling effort as large scale as this study was not feasible. Therefore, individuals sampled in this study were categorised within the three primary life stage levels: Settlement stage, Juvenile, and Adult (Table 2.3). Settlement stage ends with a full fin ray complement present, the development of scales has begun, and there is a loss of larval characters, while the juvenile life stage ends with adult body proportions, pigments, and habitats (Kendall et al. 1984).

Table 2.6: Description of simplified life stages of fish species to be used for this study (Kendall et al. 1984 adapted from Nodo 2021)

Simplified Life stage	Life stage	Description of life stage
Settlement stage	Postflexion larvae	The postflexion stages 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 pelagic to the benthic environment
Juvenile	Young-of-the-year 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	Stage ends when fish have attained sexual majority (length-at-50%-maturity)
Adult	Adults	Stage begins after fish have attained sexual majority (length-at-50%-maturity)

2.4.4 Spatial Analysis

The baseline shapefiles that were used for spatial analysis in the project were provided by SANParks. However, in order to make the best use of the GIS shapefiles for this project as well as using the shapefiles for any future work which might require high-definition habitat mapping, these shapefiles were processed further. The estuary boundary lines, and habitat boundary margins were cleaned to eliminate overlapping habitats and areas with missing data. The most recent available satellite imagery with a 20cm resolution in ArcGIS Pro (V 2.8 ©ESRI) was used to clean the habitat maps and provide new high-definition GIS shapefiles which can be used for any spatial analysis-based projects in the Knysna Estuary.

CHAPTER THREE

SPATIAL ANALYSIS OF THE LITTORAL FISH ASSEMBLAGES WITHIN THE KNYSNA ESTUARY

3.1 Introduction

Globally, it is well known that shallow littoral estuarine habitats, such as seagrass beds, salt marshes, and mangroves, provide important nursery habitats for a variety of fish species (e.g. Whitefield and Pattrick 2015, Pollard et al 2017, Whitfield 2020). These environments provide limited predation risk, abundant food resources, shelter from high-energy marine environments and biological connectivity among the variety of structurally complex habitats they provide (James et al. 2019, Whitfield 2020). Submerged plant beds in estuaries provide import habitats and nurseries for fish (Whitfield 2017) while also playing host to large numbers and biomass of invertebrates (Barnes 2010).

Of the available shallow-water littoral habitats, seagrass meadows have been regarded as the most important vegetated nursery habitat in estuaries, as they often support more juvenile fish than salt marshes, mangroves, and reed beds (Whitfield 2017). In South African estuaries, *Zostera capensis* seagrass is a common vegetation type occurring in 62 of the 290 South African estuaries and is known to play host to 100 fish species from 44 families (Whitfield et al. 1989). In addition to food provision, it has been found that *Z. capensis* also supports a host of fish species that do not utilize these habitats as a food source (Pollard et al. 2017), with seagrass habitats providing refuge from predation for these species (Whitfield 2019).

Saltmarshes also provide juvenile fish with protection from predators (Leslie et al. 2017) but with wide variation in terms of elevation, topography, and position in the estuary as well as tides and climatological events leading to differences in fish assemblages associated with saltmarshes in estuaries (Whitfield 2019). Saltmarsh fish assemblages tend to be dominated by juvenile marine fish species (Paterson and Whitfield 1996, Le Quesne 2000), which move into the intertidal saltmarshes over high tidal periods. Fish make use of these salt marshes not for feeding (Leslie et al. 2017, Whitfield 2017) but rather as an escape from larger piscivorous fishes, making the saltmarshes an essential habitat within the overall habitat mosaic of estuarine habitats (Keur et al. 2019).

The littoral zone of the Knysna Estuary is associated with a dominance of *Z. capensis*, making up a total of 30 % of the estuaries' total area (Wasserman et al. 2020). These seagrass beds play an essential role for the juvenile life stages of fish species, including economically and recreationally important fishery species, such as *Rhabdosargus holubi* and *Lithognathus lithognathus* (Whitfield et al. 2021). Due to their extent and ecological importance, seagrass beds are a crucial habitat within the Knysna Estuary. Along with subtidal and intertidal *Z. capensis* beds, intertidal salt marshes found within the Knysna Estuary have high national conservation importance (Adams et al. 2019). However, despite their importance, development and coastal squeeze threaten the saltmarsh habitats within Knysna Estuary (Claassens et al. 2020). There are also large areas of shallow intertidal sand banks that play host to *Psammogobuis knysnaensis* and *Heteromycteris capensis*. In contrast, the extensive intertidal mudbanks play host to *Solea turbynei* and the recreational fishery species *Pomadasys commersonnii* (Whitfield 2021). Littoral habitats in the Knysna Estuary have received attention due to the presence of the endangered Knysna Seahorse *Hippocampus capensis*, which has been known to utilize seagrass meadows, macroalgae, and artificial structures in littoral habitats within the estuary (Whitfield et al. 2021).

To date, two available datasets have sampled the wide range of littoral habitats in the Knysna Estuary, James and Harrison (2008) and the unpublished DFFE sample set (2007-2012) (see Appendix 1). Both datasets have shown similar catches dominated by *Atherina breviceps, Gilchristella aestuaria*, *Rhabdosargus holubi,* and *Liza richardsonii*. While these datasets have provided a much-needed description of the littoral fish assemblage in the estuary, neither has provided any information regarding key hotspots necessary for conservation planning in the system. Such information is critical in order to allow for a spatially targeted approach to the conservation of fish within the Knysna Estuary. By identifying key hotspots within the estuary, conservation efforts can be targeted at these areas most critical for the fish species utilizing the estuary.

The association of juvenile fish with shallow-water estuarine habitats means sampling these specific littoral environments is necessary to best understand the overall fish assemblages of these estuarine habitats. Seine net sampling specifically targets these littoral habitats. Seine nets are ideal for targeting shallow areas and can sample a diverse array of life stages of fish from early juveniles to adult estuarine fish, providing a thorough representation of the fish assemblage within estuarine littoral habitats.

The aim of this chapter was to sample and map the distribution and abundance of littoral fish species in the Knysna Estuary. To achieve this aim, the specific objectives of the chapter were to describe the species composition, abundance, and distribution of littoral fish in the estuary, and to identify how key hotspots may vary according to the estuarine guild and life stage of dominant species.

3.2 Materials and methods

3.2.1: Fish community sampling

The study made use of a 30 m seine net with a depth of 1.7 m, the wings of the net on either side of the bag were made up of 15 mm bar mesh with a bag made up of 5 mm bar mesh. The bottom of the net was weighted to ensure contact with the substrate was maintained. Since seine netting is most effective in shallow water due to limitations in the height of the net, the net was dropped at a distance of 20 m from the bank for shallow sites where the water depth did not exceed net height. The net was dropped closer to the bank for sites with deeper water to ensure fish could not escape over the top of the net. To best represent the overall diversity within the system, two sample periods were selected, one during summer and one during winter. The winter sample period occurred between 14 June and 18 June 2021, and the summer sample period occurred between 21 February and 23 February 2022.

All fish sampled were identified to species level, and total fish length (mm TL) was measured. At sites where large catches of a single species were made, a representative sample of 80 individuals was measured, after which individuals were counted and released.

3.2.2: Sample sites

Within each sample period, 30 sites were seined in the Knysna Estuary (Figure 3.1). Twenty-six sites were distributed throughout the system, and an additional four sites were located in the Thesen Island canals in order to better understand the utilization of this man-made structure on the fish communities in the system. The 26 sites selected throughout the estuary were based on previously selected sample sites, which were chosen to provide a representative view of the entire estuarine system (DFFE unpublished data).

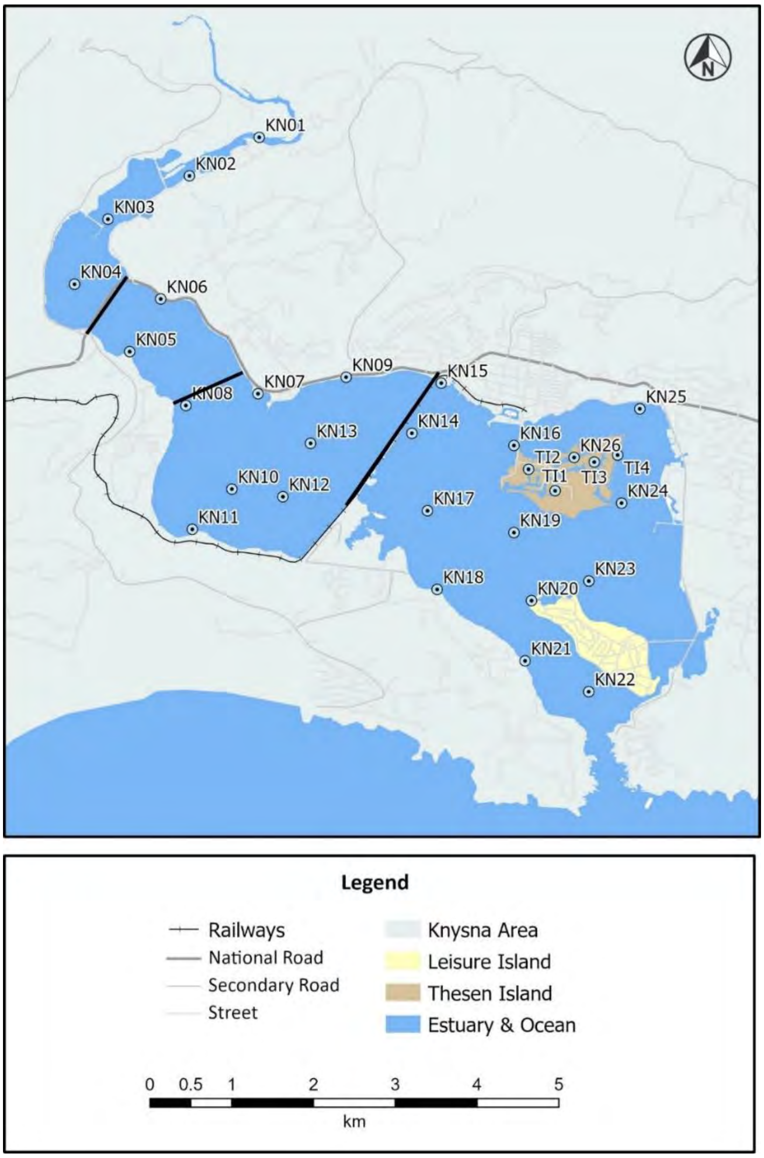


Figure 3.1: Map of the Knysna Estuary illustrating the seine net sampling sites, including the 26 sites throughout the estuary labelled KN1-KN26 and four additional sites in Thesen Island canals labelled TI1-TI4.

3.2.3: Data analysis

Catch-per-unit-effort (CPUE) of each species at each site was calculated as the total number of individuals per seine net haul averaged over the two sampling periods. All fish caught were classified into various estuarine functional guilds as defined by Potter et al. (2015) (Table 2.2, Chapter 2). GIS spatial analyses were employed to assess the spatial distribution of the littoral fish assemblage in the Knysna Estuary and to identify key hotspots in terms of estuarine guilds and life stages.

3.2.3.1: Spatial distribution by estuarine functional guild

The CPUE of each estuarine guild at each site was calculated by adding the total number of fish caught at each site in each estuarine guild and then dividing by the number of sampling periods. Due to the high variation in catches, data were fourth root transformed prior to GIS spatial analysis in ArcGIS Pro v 2.8.1. Using the fourth root transformed average CPUE, spatial distribution maps of each estuarine guild were calculated using the Kernel density tool in ArcGIS Pro. Kernel density calculation was undertaken for each estuarine guild using the average transformed catch numbers for the two-sampling period. The output of the Kernel density tool is observation/km^2 using a 10 m resolution raster, based on a search radius of 5 km and 12 nearest neighbours. Five kilometres was utilised for the search radius as it approximated half the Euclidian length of the estuary.

Due to the high density of sites around Thesen Island, the average transformed CPUE of the five sites within the Thesen Island canals, KN26, TI01, TI02, TI03, and TI04, were plotted at site KN26, which is the central Thesen Island sampling site. This ensured that the increased sampling density in the Thesen Island canals did not lead to inflated catches for the area.

3.2.3.2 Spatial distribution of dominant species by life stage

Dominant ecological species were identified as those with the highest catch from each estuarine guild. The marine straggler (MS) and estuarine and freshwater (E&F) guilds were excluded from this analysis due to the low catches of species in these guilds, with a total of five and 15 fish sampled, respectively. The dominant species, in terms of total catch within each estuarine guild, were the marine and estuarine (M&E) *Atherina breviceps*, marine-estuarine opportunist

(MEO) *Stolephorus holodon*, marine-estuarine dependent (MED) Mugilidae spp., and solely estuarine (SE) *Hyporhamphus capensis*. Important estuarine fishery species were also selected and included *Sarpa salpa, Lithognathus lithognathus, Diplodus capensis, Pomatomus saltatix, Spondyliosoma emarginatum,* and *Rhabdosargus holubi*.

The CPUE for each species by life stage were calculated for each site. As described above the average transformed CPUE of the five sites within the Thesen Island canals, KN26, TI01, TI02, TI03, and TI04, were plotted at site KN26, which is the central Thesen Island sampling site. The CPUE for each life stage were imported into the Kernel density tool, and the spatial distribution of each species per life stage was mapped as described above.

3.3 Results

3.3.1: Species composition and abundance

A total of 26 373 individuals comprising of 42 species from 20 different families were sampled during the two seine net sampling periods (Table 3.1). In terms of estuarine guilds, the total catch was divided into 14 marine estuarine opportunists (MEO) species, 10 marine-estuarine dependent (MED) species, six estuarine and freshwater (E&M) species, four marine species (MS) species, four solely estuarine (SE) species and one estuarine and freshwater (E&F) species (Table 3.1). The species with the highest catch was *Atherina breviceps,* which made up more than half of the overall catch with a total catch of 14 364 individuals (CPUE = 239.4 individuals/haul). Other species with large catches included *Stolephorus holodon* with a catch of 5 052 individuals (CPUE = 84.2 individuals/haul) and *Rhabdosargus holubi* with a total catch of 1 369 individuals (CPUE = 22.8 individuals/haul) (Table 3.1). The difficult to identify juvenile Mugilidae species consisting of four mugilid species present in the sampling effort, dominated the total Mugilidae catch with 1 663 of the total 2 592 individuals caught (Juvenile Mugilidae species CPUE = 27.0 individuals/haul).

The M&E *A. breviceps* had the highest overall catch (CPUE = 239.4 individuals/haul). The adult life stage made up most of the catch (CPUE = 232.0 individuals/haul) when compared to the settlement stage (CPUE = < 0.1 individuals/haul) and juvenile (CPUE = 7.4 individuals/haul) life stage. The MEO *S. holodon* had the largest catch in the MEO guild, with a total CPUE of 84.2 individuals/haul. In terms of life stage, juvenile *S. holodon* dominated the catch (CPUE = 60.6 individuals/haul), followed by adults (CPUE = 23.6 individuals/haul). The overall total CPUE for Mugilidae species, which comprised *Chelon dumerili* (MED), *Chelon richardsonii* (MEO), *Pseudomyxus capensis* (MED), and *Mugil cephalus* (MED), was 24.1 individuals/haul. In terms of life stage, adults were dominant (CPUE = 15.5 individuals/haul), followed by juveniles (CPUE = 8.6 individuals/haul) and a small proportion of settlement stage individuals (CPUE = < 0.1 individuals/haul).

Hyporhamphus capensis was the solely estuarine (SE) species with the largest catch, with a total CPUE of 3.0 individuals/haul. Adults dominated the catch (CPUE = 2.9 individuals/haul), with a low proportion of juveniles (CPUE = 0.1 individuals/haul) caught. *Diplodus capensis*, a MEO fishery species, had a total CPUE of 2.5 individuals/haul and was dominated by juveniles (CPUE = 2.1 individuals/haul) followed by settlement stage (CPUE = 0.4 individuals/haul) and adult stage (CPUE = <0.1 individuals/haul). *Lithognathus lithognathus* is a MED fisheries species that had a total CPUE of 0.9 individuals/haul, all of which were juveniles. *Pomatomus saltatrix*, a MEO fishery species, had a CPUE of 0.9 individuals/haul, all of which were juveniles. *Rhabdosargus holubi,* an important fishery species, fell within the MED guild and had a total CPUE of 24.1 individuals/haul. This species catch was dominated by juveniles (CPUE = 19.5 individuals/haul) followed by adults (CPUE = 3.2 individuals/haul) and settlement stage (CPUE = 0.4 individuals/haul). *Sarpa salpa* is a MEO fishery species with a total CPUE of 2.5 individuals/hauls. The catch was dominated by juveniles (CPUE = 1.8 individuals/haul) followed by adults (CPUE = 0.8 individuals/haul). *Spondyliosoma emarginatum* a MEO fishery species, had a total CPUE of 5.5 individuals/haul and was dominated by juveniles (CPUE = 5.2 individuals/haul) followed by settlement stage (CPUE = 0.3 individuals/haul).

Table 3.1: Species composition by family and estuarine guild (as per Whitfield 2019), total number of individuals, percentage contribution of each species to total catch, mean catch-per-unit-effort (CPUE), total fish length (mean and range TL mm), mean CPUE and percentage contribution of total catch by life stage, estuarine region (FS=full system, MB=marine bay, L=lagoon, LE=lower estuary, UE=upper estuary) of species sampled using a seine net in the Knysna Estuary during June 2021 and February 2022.

Family	Species	Number of individuals (% of total catch)	CPUE	Mean length (range) mm	Settlement CPUE (% species catch)	Juveniles CPUE (% species catch)	Adult CPUE (% species catch)	Estuarine region
Estuarine and freshwater (E&F)								
Gobiidae	*Glossogobius callidus*/ River Goby	25 (0.1)	0.4	55 (27-94)		<0.1 (4)	0.4 (96)	L&MB
Solely estuarine (SE)								
Clupeidae	*Gilchristella aestuaria*/ Estuarine roundherring	39 (0.1)	0.6	49 (85-32)			0.6 (100)	LE-MB
Clinidae	*Clinus spatulatus*/ Estuary klipvis	168 (0.6)	2.8	60 (34-106)		1.9 (66.7)	0.9 (33.3)	LE-MB
Hemiramphidae	*Hyporhamphus capensis*/ Cape halfbeak	182 (0.7)	3	112 (38-158)		0.1 (3.3)	2.9 (96.7)	FS
Syngnathidae	*Hippocampus capensis*/ Knysna seahorse	7 (<0.1)	0.1	55 (21-82)		0.1 (57.1)	0.1 (42.9)	L&MB
Marine and estuarine (M&E)								
Atherinidae	*Atherina breviceps*/ Cape silverside	14 364 (54.5)	239.4	52 (28-97)	<0.1 (<0.1)	7.4 (3.1)	232 (96.9)	FS
Clinidae	*Clinus superciliosus*/ Super klipvis	498 (1.9)	8.3	59 (26-110)		1.6 (18.7)	6.8 (81.3)	LE-MB
Gobiidae	*Caffrogobius gilchristi*/ Prison goby	134 (0.5)	2.2	43 (30-96)		1.8 (81.3)	0.4 (18.7)	FS
Gobiidae	*Caffrogobius nudiceps*/ Barehead goby	120 (0.5)	2	58 (30-119)		0.6 (28.3)	1.4 (71.7)	FS
Gobiidae	*Psammogobius knysnaensis*/ Knysna sandgoby	291 (1.1)	4.85	44 (24-98)		0.1 (2.8)	4.7 (97.3)	FS
Syngnathidae	*Syngnathus temmincki*/ Longsnout pipefish	38 (0.1)	0.6	152 (58-220)		0.1 (13.2)	0.6 (86.8)	FS
Marine-estuarine dependent (MED)								
Carangidae	*Lichia amia*/ Leervis	4 (<0.1)	0.1	225 (112-321)		0.1 (100.0)		L&MB

Monodactylidae	*Monodactylus falciformis*/ Oval moony	82 (0.3)	1.4	56 (19-200)	0.2 (13.4)	1 (73.2)	0.2 (13.4)	FS
Mugilidae	*Chelon dumerili*/ Groovy mullet	79 (0.3)	1.3	183 (45-365)		0.8 (58.2)	0.6 (41.8)	FS
Mugilidae	*Pseudomyxus capensis*/ Freshwater mullet	33 (0.1)	0.6	275 (110-354)		0.1 (15.2)	0.5 (84.9)	UE
Mugilidae	Mugilidae spp/ Mullet	1 633 (6.2)	27	51 (15-101)	0.6 (2.1)	26.4 (97.1)		LE-MB
Mugilidae	*Mugil cephalus*/ Flathead mullet	5 (<0.1)	0.1	134 (125-145)		0.1 (100.0)		MB
Soleidae	*Heteromycteris capensis*/ Cape sole	224 (0.9)	3.7	49 (20-85)	<0.1 (0.9)	3.6 (97.3)	0.1 (1.8)	L&MB
Soleidae	*Solea turbynei*/ Blackhand sole	26 (0.1)	0.4	49 (23-91)		0.3 (61.5)	0.2 (34.6)	FS
Sparidae	*Lithognathus lithognathus*/ White Steenbras	56 (0.2)	0.9	179 (54-461)		0.9 (100.0)		FS
Sparidae	*Rhabdosargus holubi*/ Cape Stumpnose	1 369 (5.2)	22.8	86 (15-355)	0.4 (0.5)	19.5 (85.5)	3.2 (14.0)	FS
Marine-estuarine opportunist (MEO)								
Carangidae	*Alectis indicus*/ Indian threadfish	1 (<0.1)	<0.1	111		<0.1 (100.0)		LE
Engraulidae	*Stolephorus holodon*/ Thorny anchovy	5 052 (19.2)	84.2	46 (32-65)		60.6 (72.0)	23.6 (28.0)	FS
Haemulidae	*Pomadasys olivaceus*/ Piggy	2 (<0.1)	<0.1	72 (28-85)		<0.1 (100.0)		MB
Hemiramphidae	*Hemiramphus far*/ Spotted halfbeak	332 (1.3)	5.5	119 (50-184)	0.2 (4.2)	5.3 (95.8)		LE-MB
Mugilidae	*Chelon richardsonii*/ Southern Mullet	812 (3.1)	13.5	156 (21-395)	0.1 (0.5)	9.23 (68.2)	4.2 (31.3)	FS
Pomatomidae	*Pomatomus saltatrix*/ Shad	55 (0.2)	0.9	123 (83-155)		0.9 (100.0)		LE-MB
Sparidae	*Diplodus hottentotus*/ Zebra	21 (0.1)	0.4	94 (54-121)		0.4 (100.0)		LE-MB
Sparidae	*Diplodus capensis*/ Blacktail	149 (0.6)	2.5	58 (12-313)	0.4 (16.8)	2.1 (82.6)	<0.1 (0.7)	LE-MB
Sparidae	*Lithognathus mormyrus*/ Sand steenbras	23 (0.1)	0.4	93 (30-184)		0.3 (82.6)	0.1 (17.4)	L&MB
Sparidae	*Rhabdosargus globiceps*/ White stumpnose	30 (0.1)	0.5	33 (21-94)	0.1 (23.3)	0.4 (76.7)		MB
Sparidae	*Sarpa salpa*/ Strepie	151 (0.6)	2.5	134 (52-286)		1.8 (69.5)	0.8 (30.5)	LE-MB

Sparidae	*Spondyliosoma emarginatum*/ Steenjie	327 (1.2)	5.5	65 (22-147)	0.3 (4.6)	5.2 (95.4)		L&MB
Sphyraenidae	*Sphyraena jello*/ Pickhandle barracuda	1 (<0.1)	<0.1	62				MB
Tetraodontidae	*Geneion honckenii*/ Evileye blaasop	23 (0.1)	0.4	90 (41-195)		0.3 (73.9)	0.1 (26.1)	FS
Marine stragglers (MS)								
Carangidae	*Trachurus trachurus*/ Horse mackerel	5 (<0.1)	0.1	97 (76-122)		0.1 (100.0)		MB
Cynoglossidae	*Cynoglossus capensis*/ Sand toungefish	2 (<0.1)	<0.1	49 (43-55)		<0.1 (100.0)		MB
Dasyatidae	*Dasyatis chrysonota*/ Blue stingray	1 (<0.1)	<0.1	220	<0.1 (100.0)			MB
Fistulariidae	*Fistularia commersonii*/ Smooth Flutemouth	1 (<0.1)	<0.1	167		<0.1 (100.0)		L
Ostraciidae	*Ostracion cubicus*/ Yellow boxfish	1 (<0.1)	<0.1	15	<0.1 (100.0)			MB

In terms of estuarine utilisation, the percentage composition of the total catch was dominated by the M&E guild in the lower estuary (93%), lagoon (52%) and marine bay (78%) region (Figure 3.2). The upper estuary, however, was dominated by MEO species, making up 93% of the total catch for this region (Figure 3.2). Of the remaining guilds, MED and SE species were present in every region, while the E&F and MS species were only present in the lagoon and marine bay region (Figure 3.2).

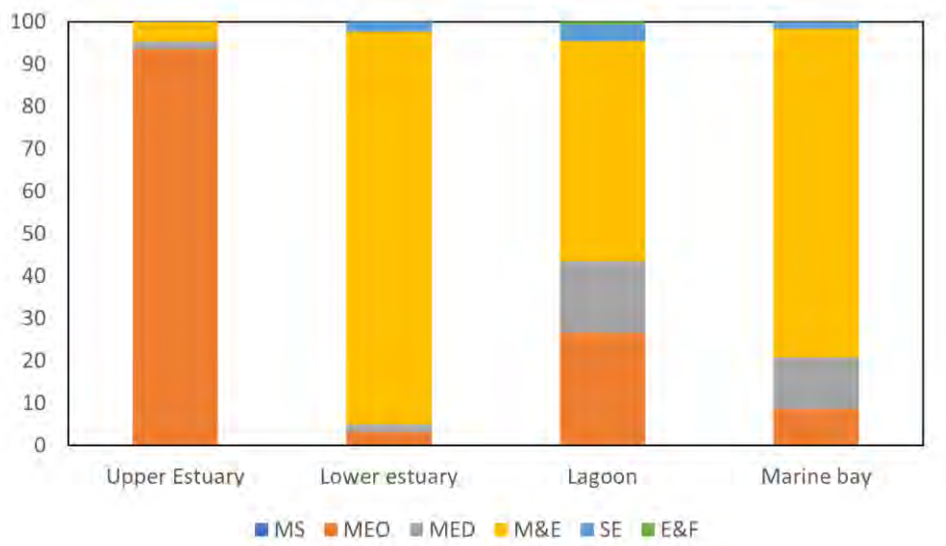


Figure 3.2: Percentage composition of estuarine guilds (MS = marine stragglers, MEO = marine-estuarine opportunists, MED = marine-estuarine dependents, M&E = marine and estuarine, SE = solely estuarine, E&F = estuarine and freshwater) to the total catch-per-unit effort sampled using a seine net in the different regions of the Knysna Estuary during June 2021 and February 2022.

In terms of life stage, adult specimens dominated the E&F, SE and M&E guilds, while the juvenile life stages were dominant in the MED, MEO and MS guilds (Figure 3.2). The settlement stage comprised small proportions of the MED, MEO and MS catch (Figure 3.2).

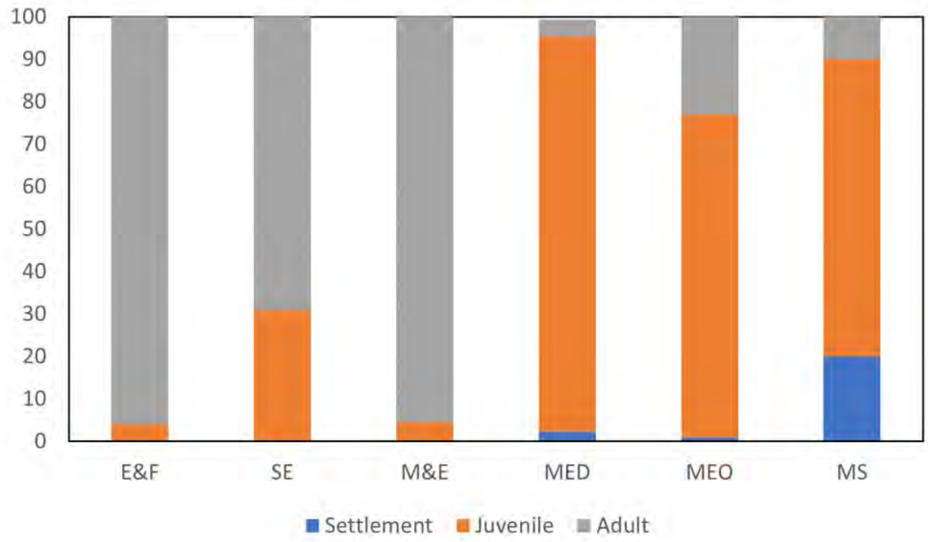


Figure 3.3: Percentage composition of life stage per estuarine guild (E&F = estuarine and freshwater, SE = solely estuarine, M&E = marine and estuarine, MED = marine-estuarine dependents, MEO = marine-estuarine opportunists, MS = marine stragglers) to the total catch-per-unit-effort sampled using a seine net in the Knysna Estuary during June 2021 and February 2022.

3.3.2 Spatial distribution of estuarine guilds

The highest catches of SE, M&E, MED, MEO and MS guilds were observed in the marine bay section, with a density hotspot in this region for these species. Estuarine and freshwater (E&F) species showed the highest catch in the lagoon region of the system (Figure 3.4). The estuarine and freshwater guild exhibited a widespread hotspot throughout the lagoon region of the system, with a secondary hotspot in the marine bay region in the Ashmead channel below Thesen Island (Figure 3.4). The solely estuarine guild exhibited a broad distribution throughout the estuary with an extended hotspot throughout the system's marine bay and lagoon region. The guild showed low densities in the mouth region of the system and at the top of the estuary in the upper estuary region (Figure 3.4). The marine and estuarine guild exhibited a broad distribution throughout the estuary with a distinct hotspot in the marine bay and lagoon region of the estuary; the hotspot extends from the head of Thesen Island in the marine bay region to below the railway bridge. *Atherina breviceps* is mainly responsible for the distribution of this guild making up 14 364 individuals of the total 14 683 individuals from this guild (Figure 3.4).

The marine-estuarine dependent guild exhibited a broad distribution throughout the estuary with a hotspot in the marine bay region of the system; the hotspot extends from the head of Leisure Island to Thesen Island, covering the Thesen Island canals. This guild's catch was dominated by the Mugilidae species (*C. dumerili, M. cephalus,* juvenile Mugilidae and *P. capensis*), making up 864 individuals of the total 1 237 specimens sampled (Figure 3.4). The MEO guild exhibited widespread distribution throughout the estuary, with three distinct hotspots, with one hotspot in the marine bay from the head of Leisure Island to Thesen Island; a hotspot in the Lagoon region from the railway bridge throughout much of the lagoon region and a hotspot in the upper estuary region of the system above the N2 bridge. This hotspot was comprised mainly of *S. holodon* which made up 5 000 individuals out of the total 6 581 for this guild (Figure 3.4). The low catches for the MS guild resulted in no clear hotspot, with only three specimens sampled. A single specimen was sampled in the Thesen Island Canal and in the Ashmead Channel in the marine bay region, with a specimen sampled in the lagoon region (Figure 3.4).

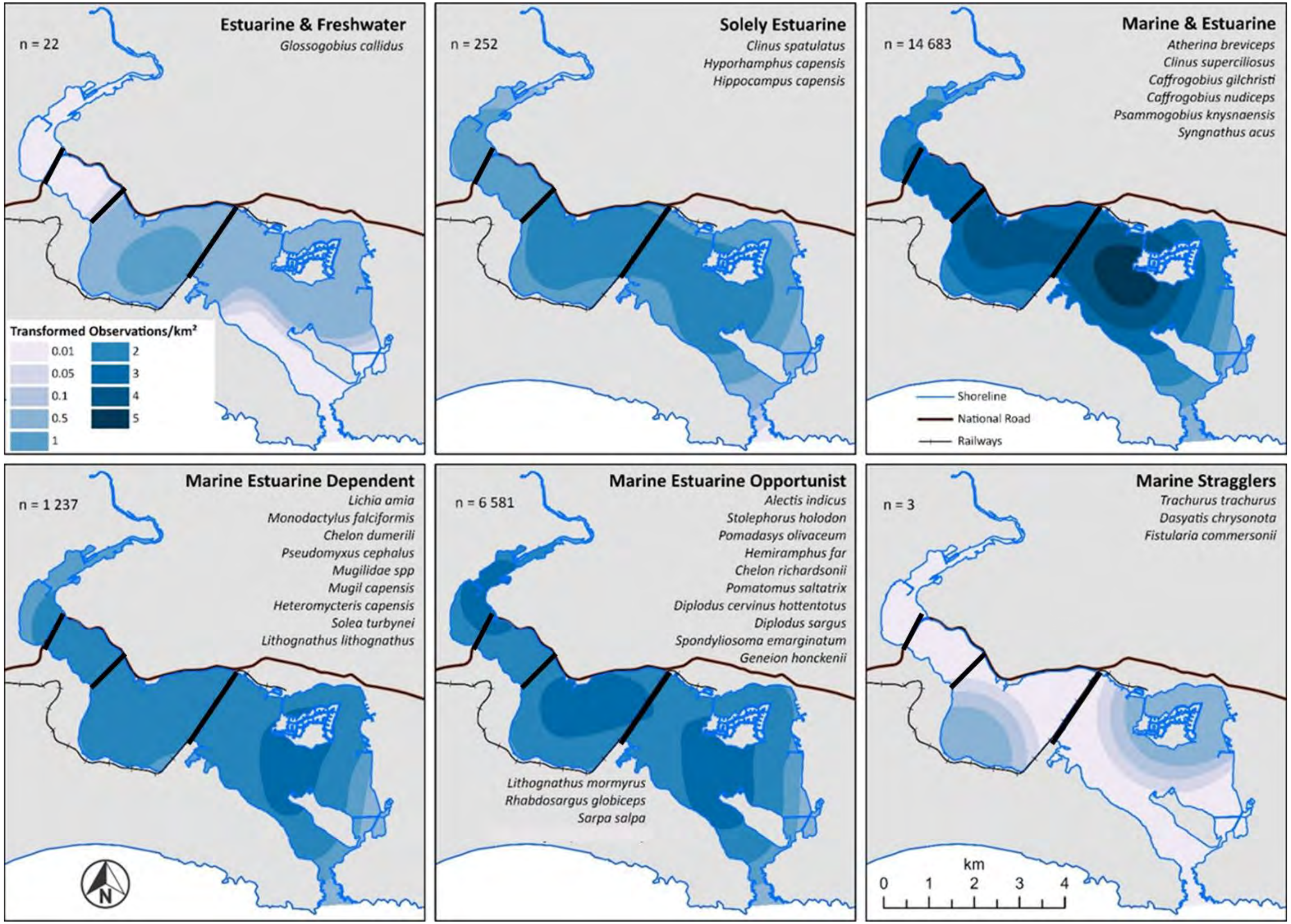


Figure 3.4: Kernel density spatial distribution maps of the CPUE over two sampling trips (June 2021 and February 2022) for seine net catches within the Knysna Estuary categorised by their estuarine guilds (as defined by Whitfield 2019), with total catches indicated (n). Data were square root transformed prior to GIS analysis

3.3.3 Spatial distribution of dominant species and important fishery species by life stage

Dominant species

Atherina breviceps exhibit a distinct hotspot in the marine bay region of the system near the head of Thesen Island, with an estimated CPUE of 1775 individuals/haul for this hotspot. This hotspot was made up almost entirely of adult *A. breviceps*, with juvenile *A. breviceps* showing an extended hotspot throughout the estuary. Low catches of settlement stage *A. breviceps* did not reflect any hotspot (Figure 3.5).

Stolephorus holodon exhibit density hotspots in one site in the upper estuary region of the estuary with a CPUE of 2 500.0 individuals/haul for this hotspot, which were representative of both juvenile (CPUE = 1 779.0 individuals/haul) and adult life stages (CPUE = 701.0 individuals/haul) (Figure 3.6).

Mugilidae spp exhibit a distinct density hotspot in the Thesen Island canals with an estimated CPUE of 128.1 individuals/haul; this was dominated by juvenile mugilidae, which make up most of the total catch. Adult Mugilidae did not reflect any distinct hotpots showing relatively consistent distribution throughout the estuary (Figure 3.7).

Hyporhamphus capensis showed density hotspots in the lower estuary region of the system (CPUE = 34.0 individuals/haul), made up of adult specimens. The low catch of juvenile *H. capensis* did not reflect any hotspots (Figure 3.8).

Fishery species

Diplodus capensis exhibit a density hotspot in the marine bay region of the system between the railway bridge and Thesen Island. Within this hotspot an estimated CPUE of 8.5 individuals/haul was made up of juveniles, settlement stage *D. capensis* were mainly along the west bank of the marine bay region, with an estimated CPUE of 9.0 individuals/haul. The single adult specimen of *D. capensis* did not reflect any adult life stage hotspot (Figure 3.9).

Juvenile *L. lithognathus* exhibit density hotspots (estimated CPUE = 5.0 individuals/haul) in the marine bay region of the estuary, between Thesen Island and Leisure Island with a secondary hotspot in the lower estuary region with an estimated CPUE of 1.25 individuals/haul (Figure 3.10).

Juvenile *P. saltatrix* exhibits a density hotspot, with an estimated CPUE of 19.5 individuals/haul, in the lagoon region of the estuary. *Pomatomix saltatrix* were also present in the marine bay region at the head of Leisure Island (Figure 3.11).

Rhabdosargus holubi exhibit a density hotspot in the marine bay region around Thesen Island, with an estimated CPUE of 51.0 individuals/hauls and a secondary hotspot with an estimated CPUE of 44.0 individuals/haul in the lagoon region of the estuary; both of these hotspots were dominated by juvenile specimens. Adult *R. holubi* specimens showed relatively consistent distribution throughout the system with a minor hotspot (CPUE = 9.0 individuals/haul) in the lagoon region of the system. The low catches of settlement stage *R. holubi* did not reflect any hotspots (Figure 3.12).

Sarpa salpa exhibited density hotspots (CPUE = 15.5 individuals/haul) in the lagoon region of the estuary, with secondary hotspots (CPUE = 10.7 individuals/haul) in the lagoon region of the estuary (Figure 3.12). Juvenile *S. salpa* exhibited a density hotspot in the lagoon region (CPUE = 11.0 individuals/haul) and in the Thesen Island Canals in the marine bay region (CPUE = 5.8 individuals/haul), while adult *S. salpa* exhibit a density hotspot at the head of Leisure Island in the marine bay region (CPUE = 10.5 individuals/haul) (Figure 3.13).

Spondyliosoma emarginatum showed density hotspots in the marine bay region of the estuary, with an estimated CPUE of 56.5 individuals/haul between Thesen and Leisure Island made of juvenile specimens. Low catches of settlement stage *S. emarginatum* did not reflect any hotspots (Figure 3.14).

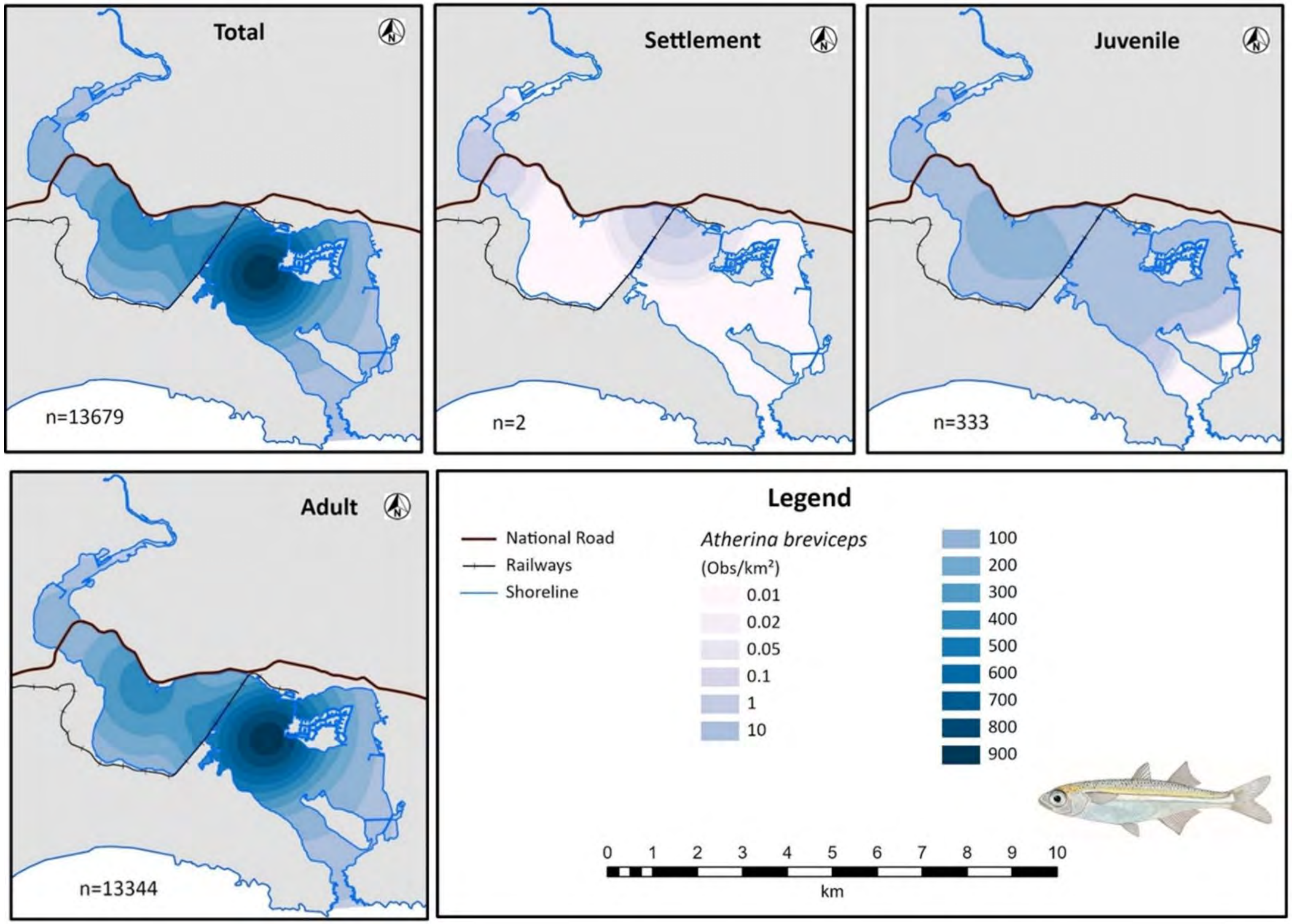


Figure 3.5: Kernel density spatial distribution maps of the catch-per-unit-effort (CPUE) over two sampling trips (June 2021 and February 2022) for the dominant estuarine and marine (E&M) species sampled in the Knysna Estuary, *Atherina breviceps*. Densities represent catch-per-unit-effort, including total, settlement, juvenile, and adult specimens sampled using a seine net. n = catch numbers.

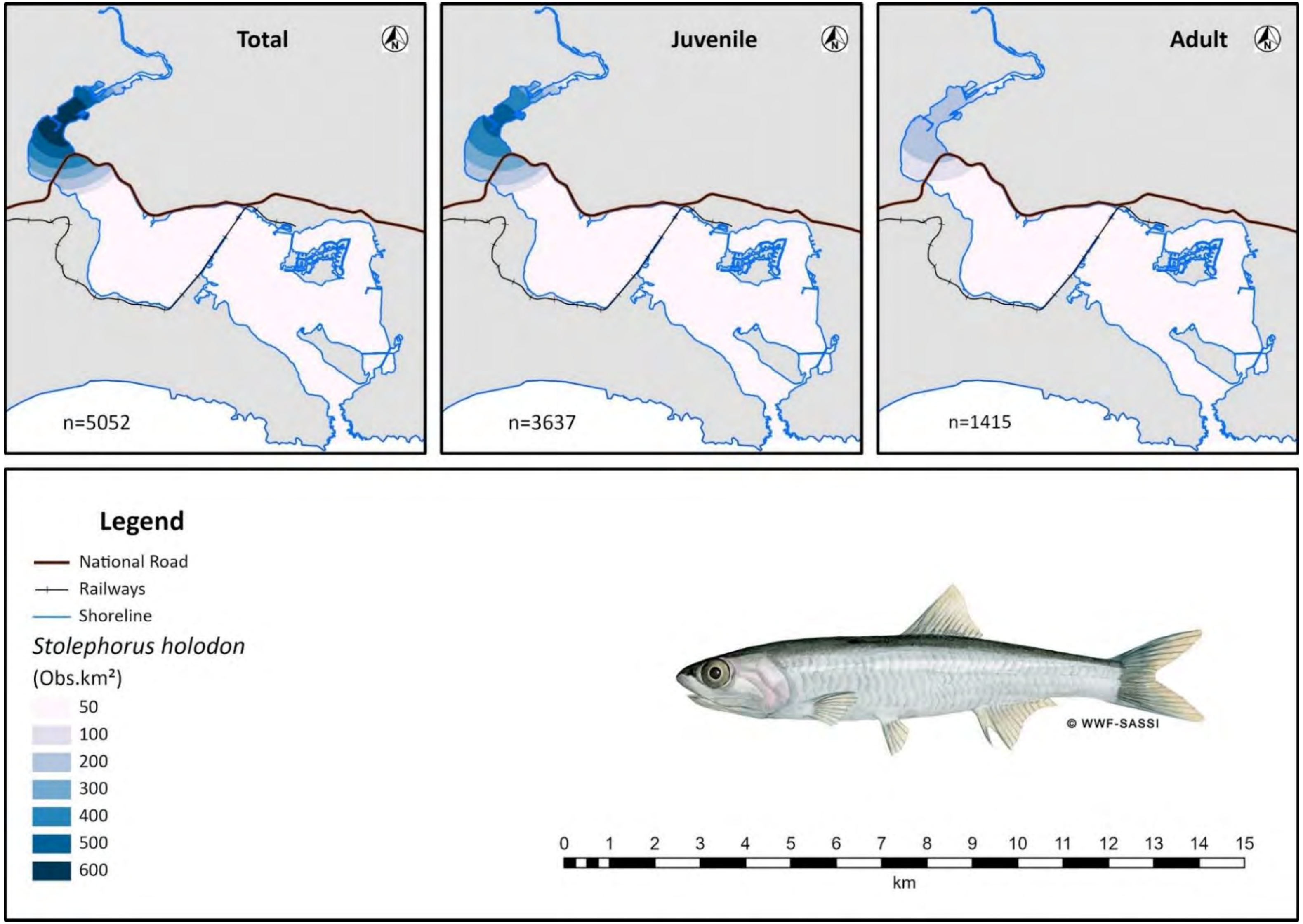


Figure 3.6: Kernel density spatial distribution maps of the catch-per-unit-effort (CPUE) over two sampling trips (June 2021 and February 2022) for the dominant marine-estuarine opportunist (MEO) species sampled in the Knysna Estuary, *Stolephorus holodon*. Densities represent catch-per-unit-effort, including total, juvenile, and adult specimens sampled using a seine net. n = catch numbers.

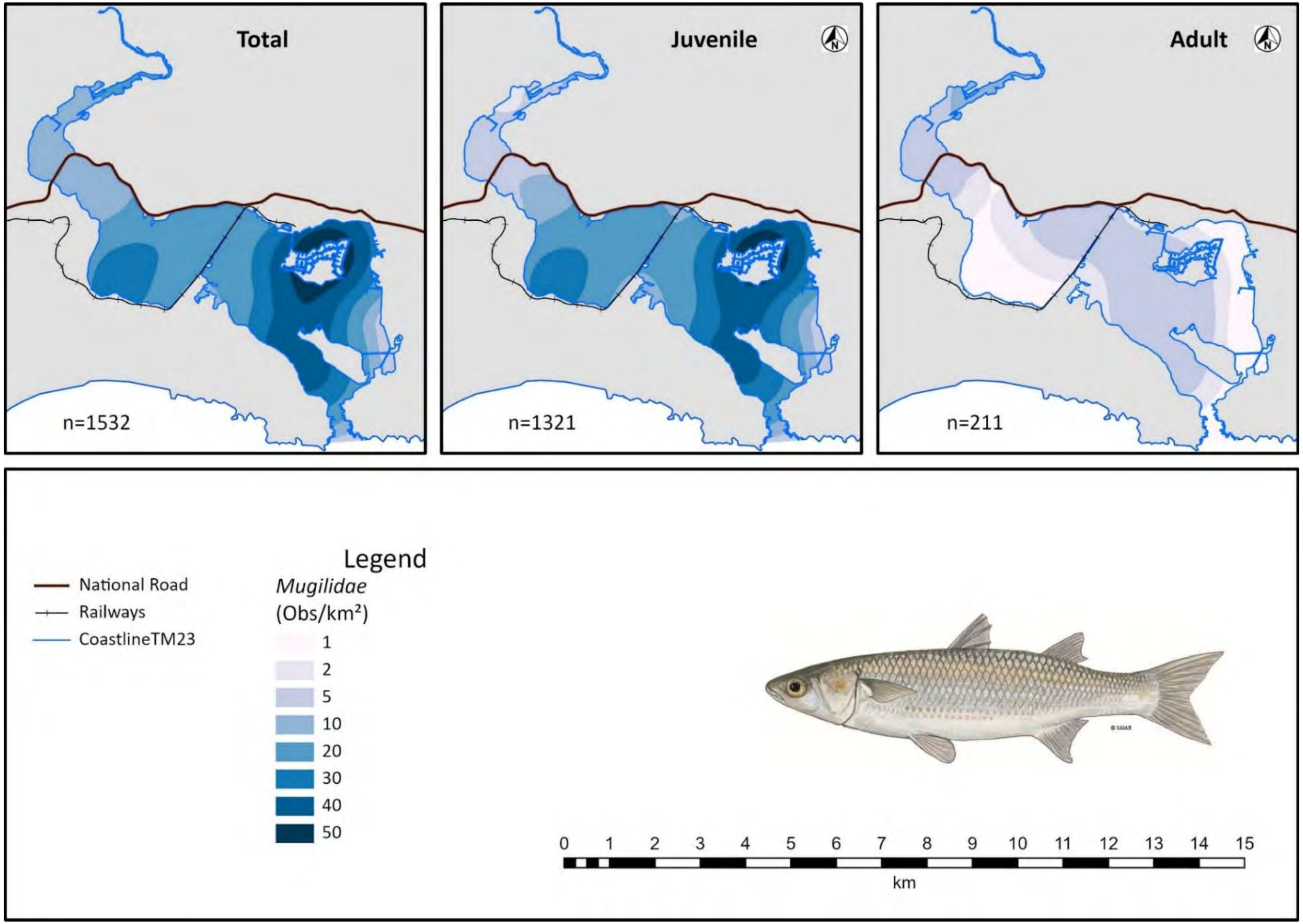


Figure 3.7: Kernel density spatial distribution maps within the Knysna Estuary of the catch-per-unit-effort (CPUE) over two sampling trips (June 2021 and February 2022) for the dominant marine-estuarine dependant (MED) family sampled in the Knysna Estuary, the Mugilidae species. Densities represent catch-per-unit-effort, including total, juvenile, and adult specimens sampled using a seine net. n = catch numbers.

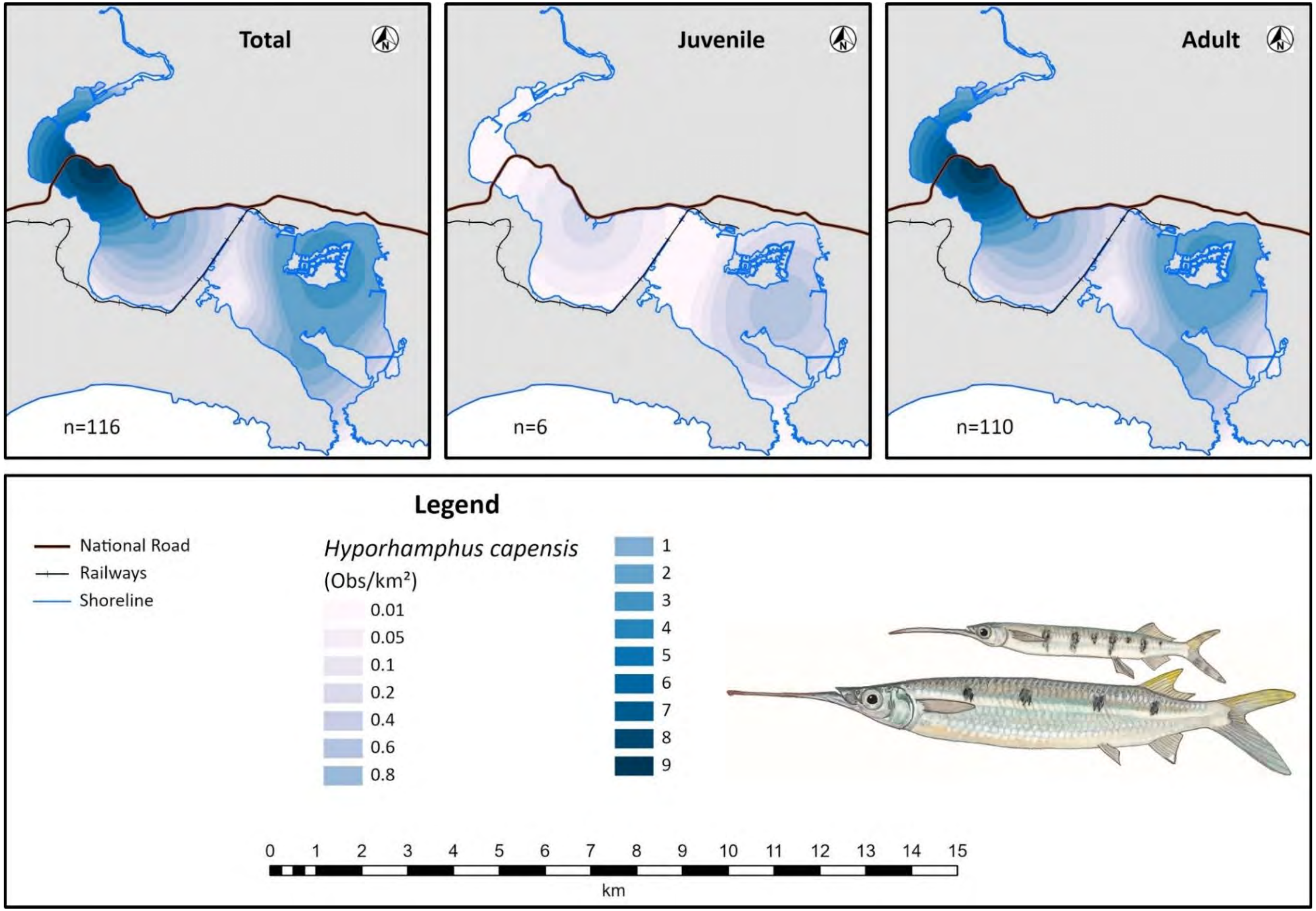


Figure 3.8: Kernel density spatial distribution maps of the catch-per-unit-effort (CPUE) over two sampling trips (June 2021 and February 2022) for the dominant solely estuarine (SE) species sampled in the Knysna Estuary, *Hyporhamphus capensis*. Densities represent catch-per-unit-effort, including total, settlement, juvenile, and adult specimens sampled using a seine net. n = catch numbers.

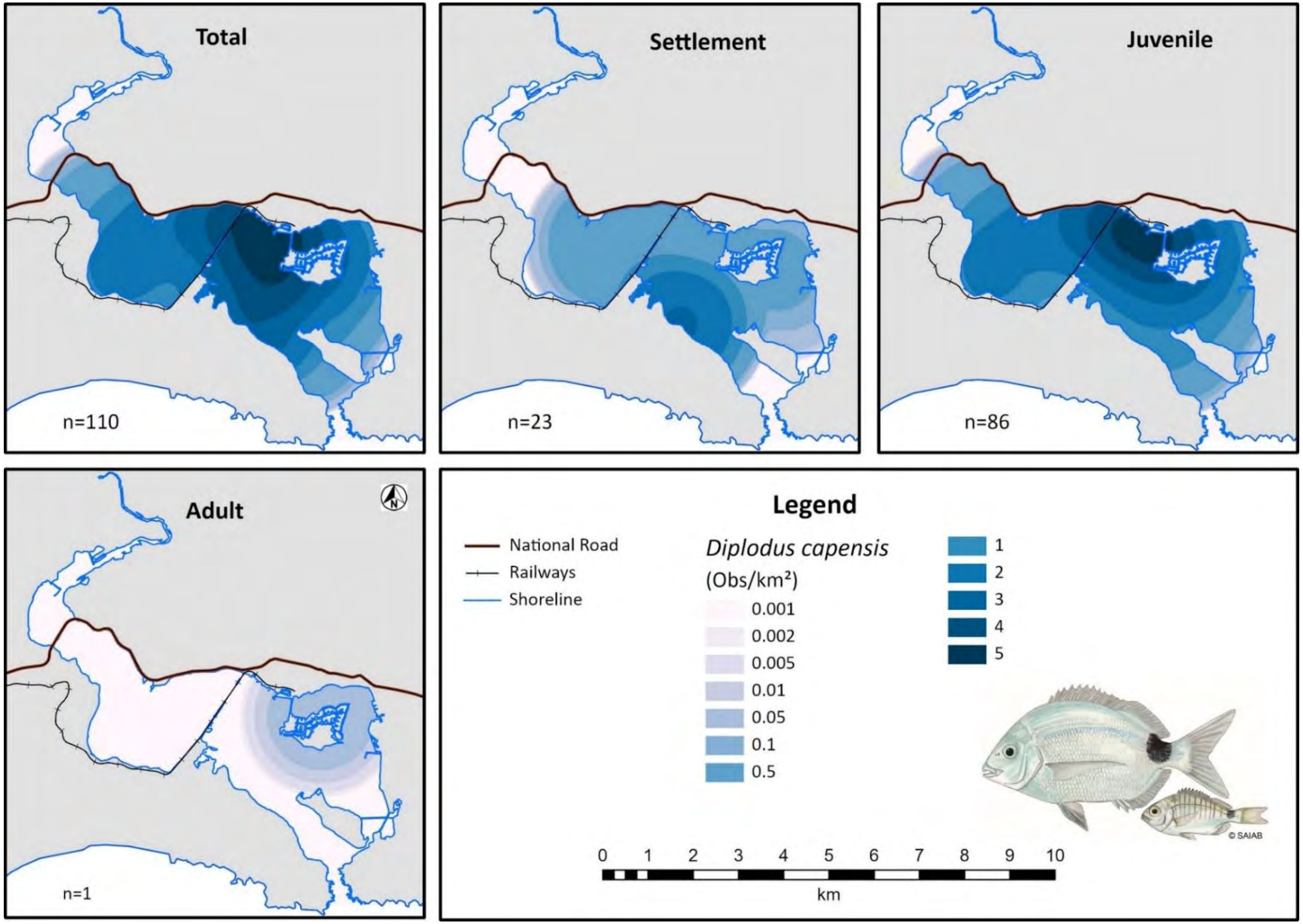


Figure 3.9: Kernel density spatial distribution maps of the catch-per-unit-effort (CPUE) over two sampling trips (June 2021 and February 2022) for a fishery marine-estuarine opportunist (MEO) species sampled in the Knysna Estuary, *Diplodus capensis*. Densities represent catch-per-unit-effort, including total, settlement, juvenile, and adult specimens sampled using a seine net. n = catch numbers.

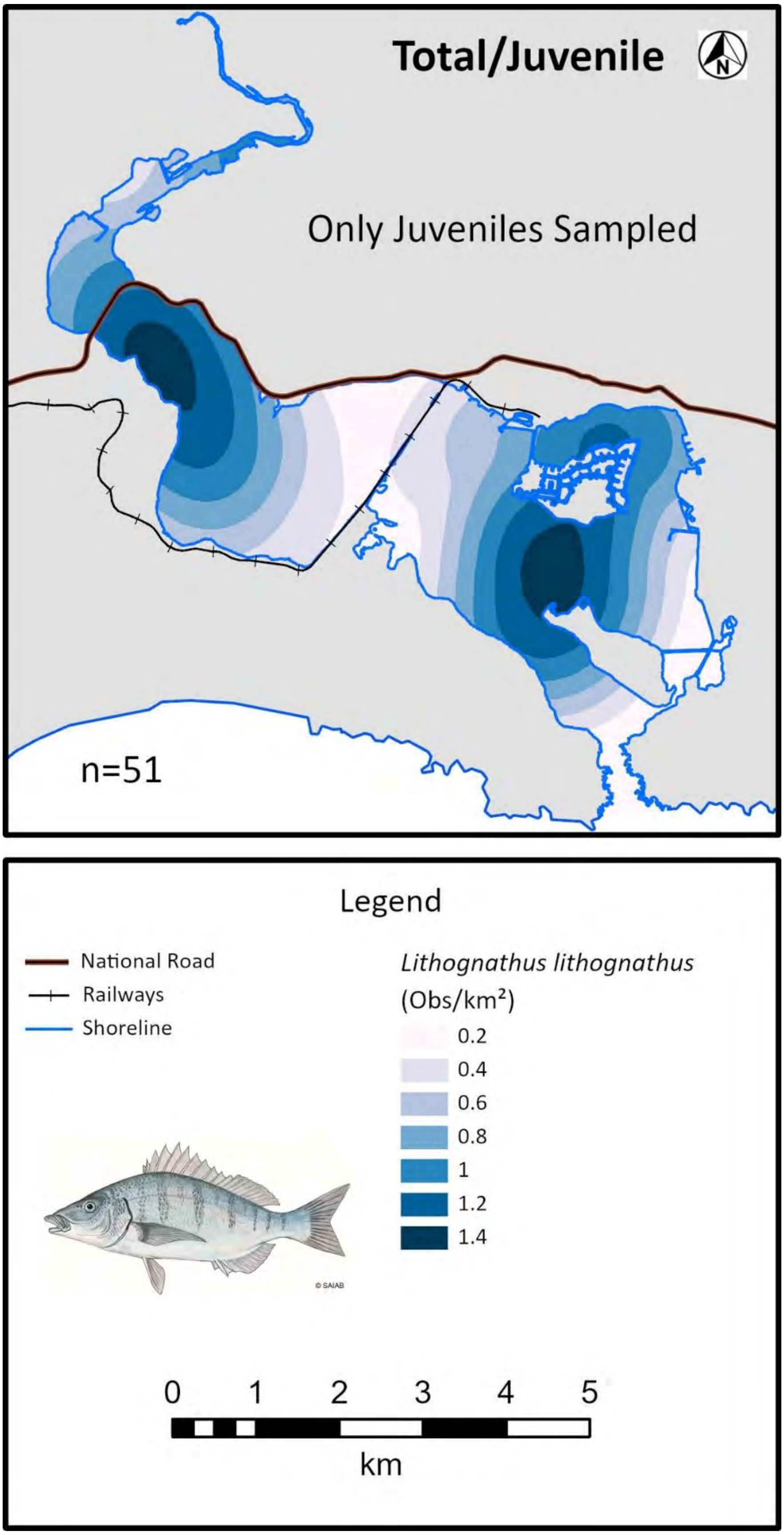


Figure 3.10: Kernel density spatial distribution maps of the catch-per-unit-effort (CPUE) over two sampling trips (June 2021 and February 2022) for a fishery marine-estuarine dependent (MED) species sampled in the Knysna Estuary, *Lithognathus lithognathus.* Densities represent catch-per-unit-effort, including total, settlement, juvenile, and adult specimens sampled using a seine net. n = catch numbers.

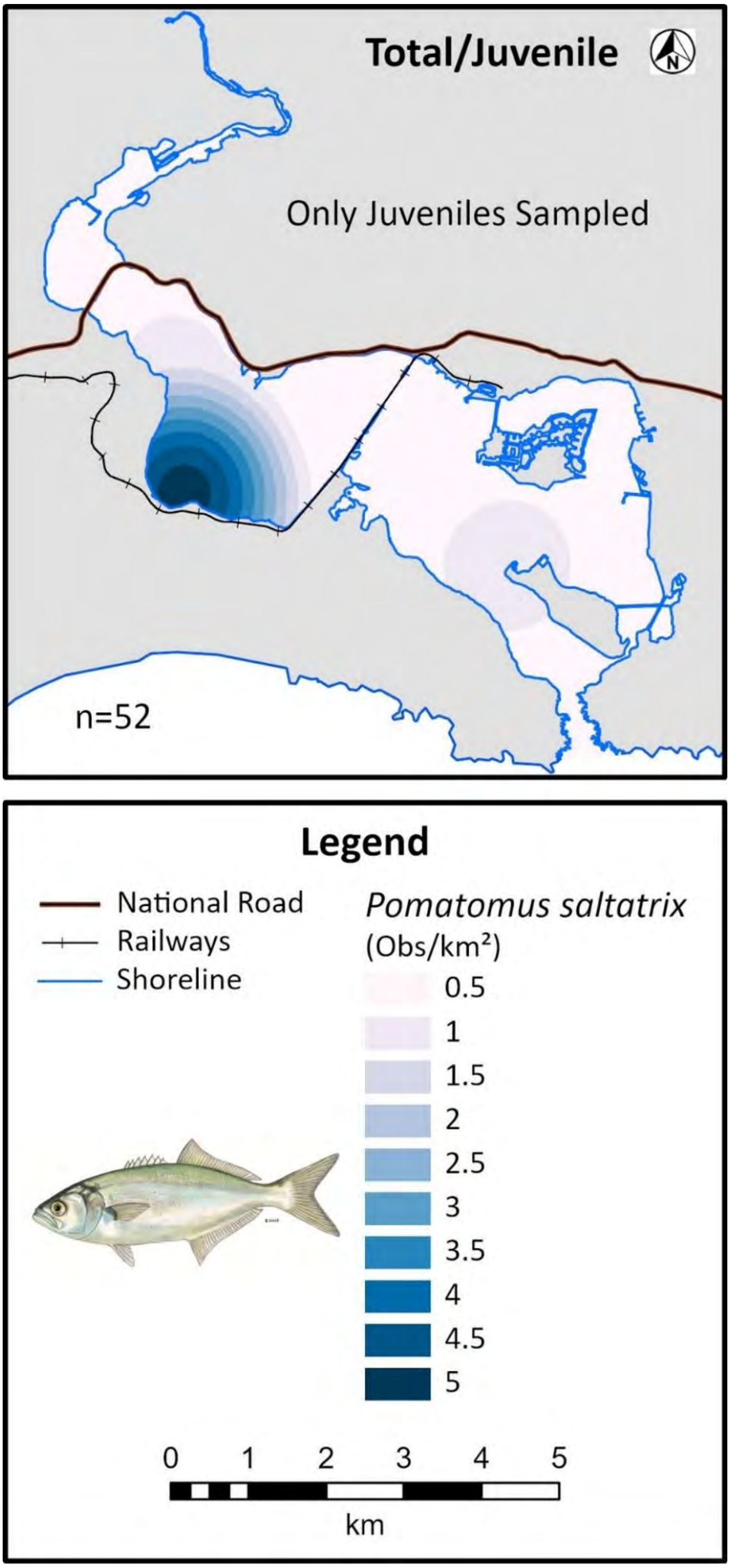


Figure 3.11: Kernel density spatial distribution maps of the catch-per-unit-effort (CPUE) over two sampling trips (June 2021 and February 2022) for a fishery marine-estuarine opportunist (MEO) species sampled in the Knysna Estuary, *Pomatomus saltatrix*. Densities represent catch-per-unit effort including only juvenile specimens sampled using a seine net. n = catch numbers.

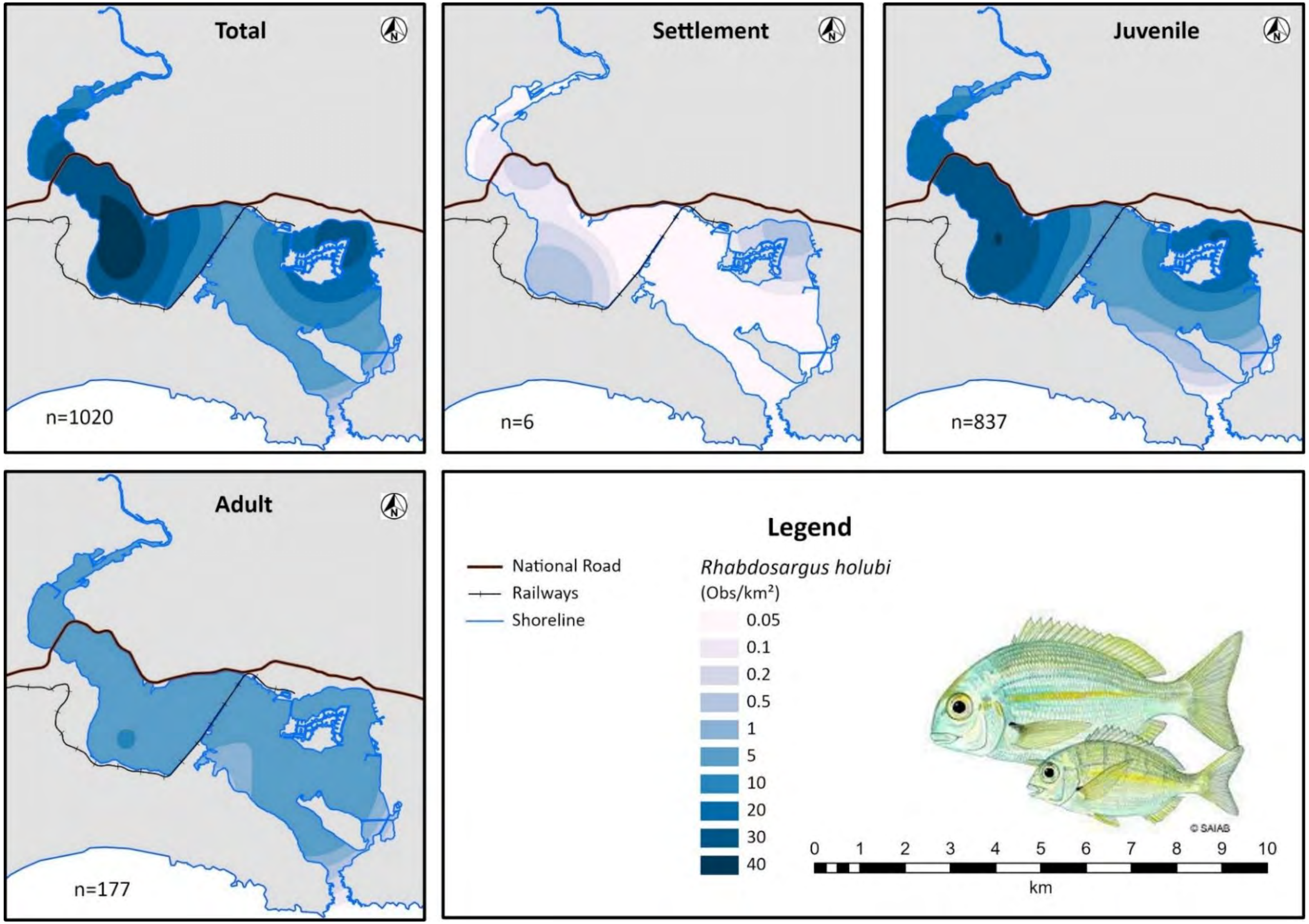


Figure 3.12: Kernel density spatial distribution maps of the catch-per-unit-effort (CPUE) over two sampling trips (June 2021 and February 2022) for a fishery marine-estuarine dependent (MED) species sampled in the Knysna Estuary, *Rhabdosargus holubi*. Densities represent catch-per-unit-effort, including total, settlement, juvenile, and adult specimens sampled using a seine net. n = catch numbers.

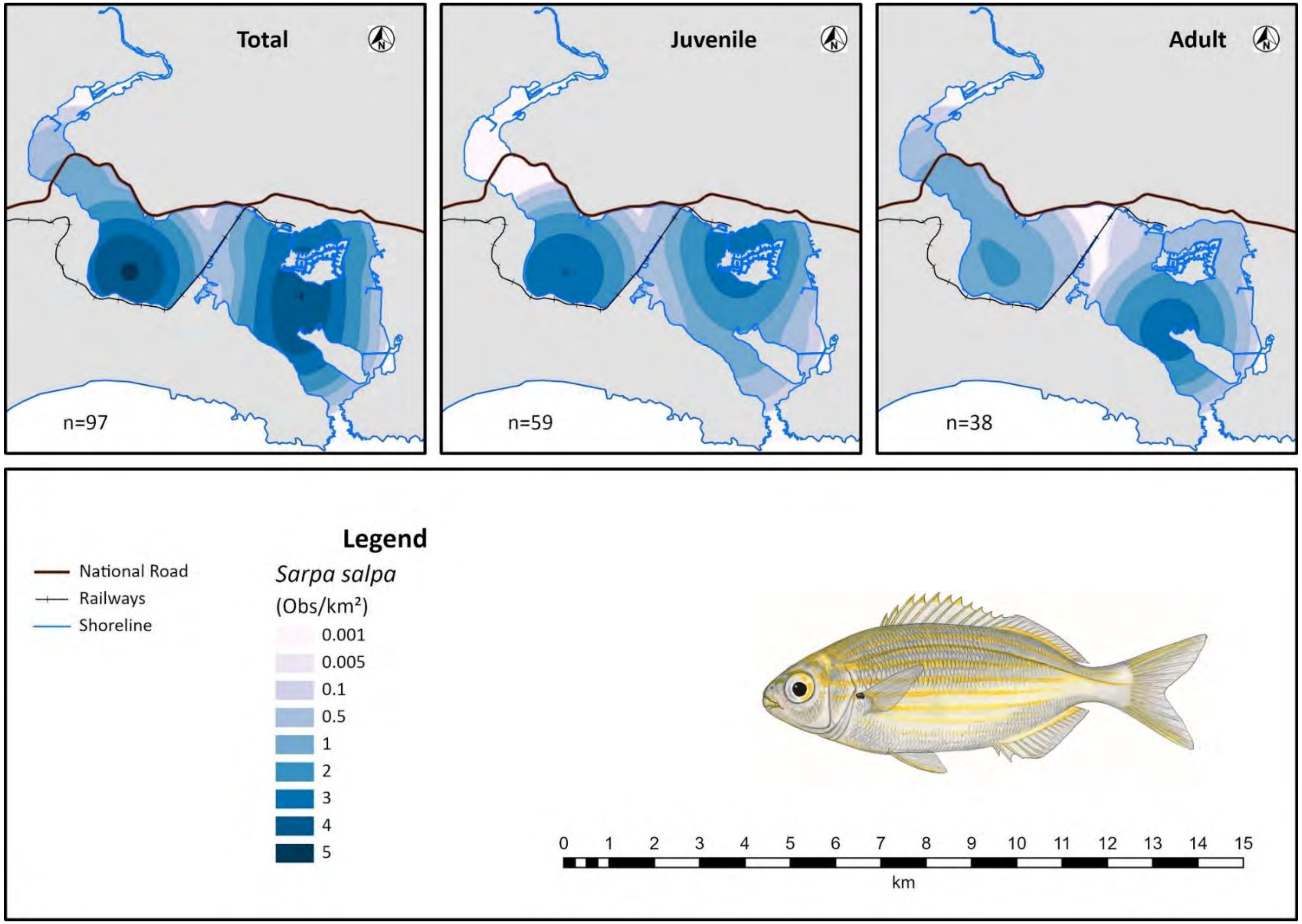


Figure 3.13: Kernel density spatial distribution maps of the catch-per-unit-effort (CPUE) over two sampling trips (June 2021 and February 2022) for a fishery marine-estuarine opportunist (MEO) species sampled in the Knysna Estuary, *Sarpa salpa*. Densities represent catch-per-unit-effort, including total, juvenile, and adult specimens sampled using a seine net. n = catch numbers.

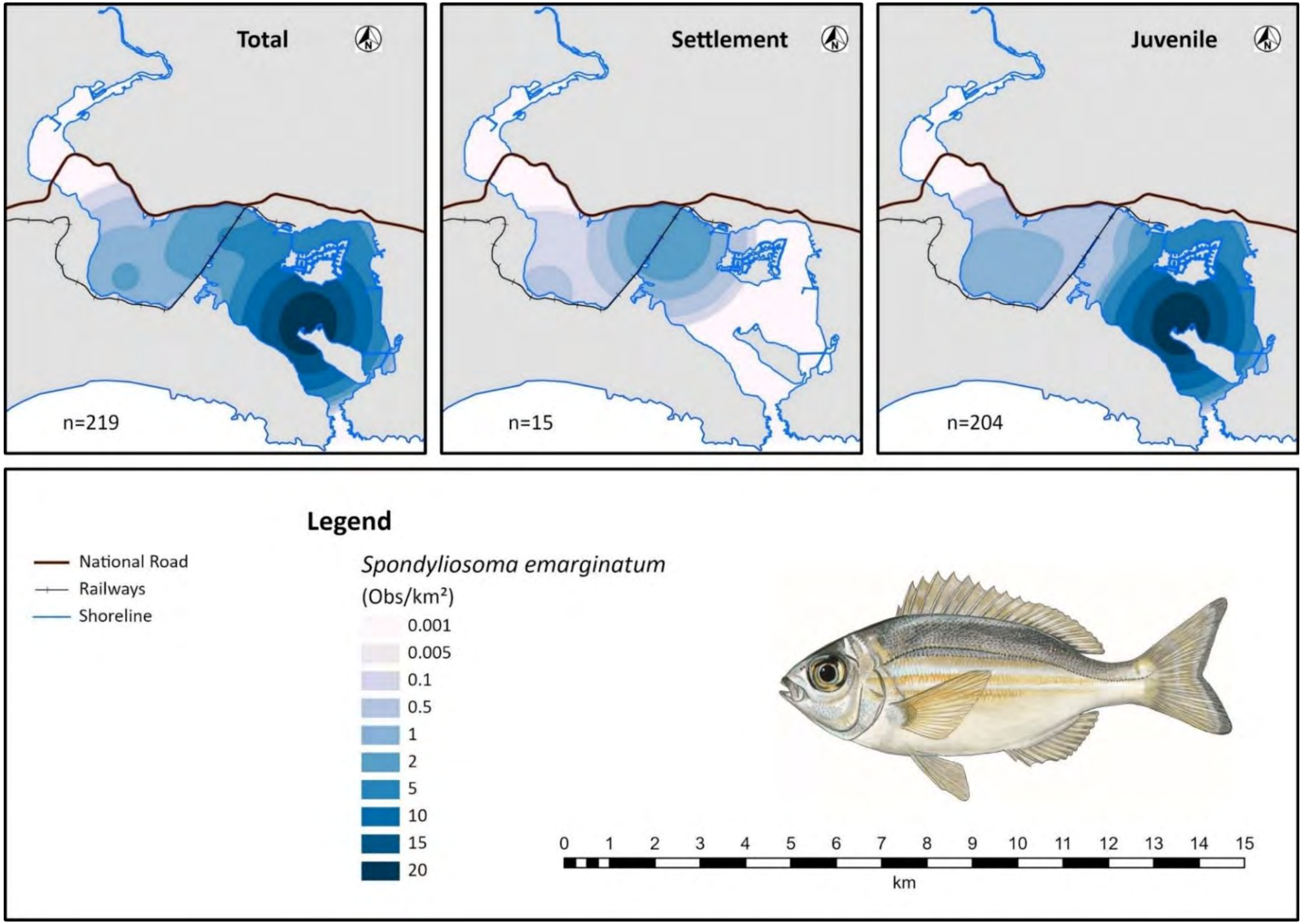


Figure 3.14: Kernel density spatial distribution maps of the catch-per-unit-effort (CPUE) over two sampling trips (June 2021 and February 2022) for a marine-estuarine opportunist (MEO) fishery species sampled in the Knysna Estuary, *Spondyliosoma emarginatum*. Densities represent catch-per-unit-effort, including total, settlement and juvenile specimens sampled using a seine net. n = catch numbers.

3.4 Discussion

Identification of key hotspot areas within estuaries is essential for effective spatial conservation planning (e.g. Prestrelo and Vianna 2016, Costa et al. 2016, Lagabrielle et al. 2018, Costa et al. 2022), with spatial distribution allowing a better understanding of how fish species are making use of estuaries, and highlighting potential important areas for fish conservation. The marine bay region of the system showed widespread hotspots for most estuarine guilds, with additional hotspots in the lagoon region of the system for E&F, M&E and MEO. The SE and MED guilds only showed hotspots in the marine bay. All the key species showed diverse hotspots throughout the marine bay, with the exception of *Stolephorus holodon,* which showed a hotspot in the upper estuary region of the system and *Pomatomus saltatrix*, which showed a hotspot in the lagoon region. The hotspot for *P. saltatrix* was also a secondary hotspot for other fishery species *L. lithognathus, R. holubi* and *S. salpa*.

Hotspots were identified in the marine bay region of the estuary for several species. The fishery species *Lithognathus lithognathus, Sarpa salpa* and *Spondliosoma emarginatum* all showed similar marine bay hotspots between Thesen Island and Leisure Island. The marine bay hotspot identified for *D. capensis* on the east bank did not overlap with the other fishery species hotspots identified in the central region of the marine bay and the lagoon region. The key Sparidae species identified, *Diplodus capensis, Lithognathus lithognathus, Rhabdosargus holubi, Sarpa salpa,* and *Spondylisoma emarginatum* all showed relatively condensed hotspots focussed around *Z. capensis* habitats in the marine bay region. *Diplodus capensis* was especially notable for this, with all hotspots for these life stages being in *Z. capensis* beds in the marine bay region of the system. *Spondylisoma emarginatum* is an interesting species as it is a marine-associated taxon which has been noted regularly in the Knysna Estuary, especially during summer in the lower reaches of the system (Whitfield and Kok 1992). The Mugilidae species sampled during this effort did not show a strong association with *Z. capensis*. Instead, they showed a relatively widespread distribution through the system's marine bay and lagoon regions, with higher abundances occurring in the Thesen Island canals, which are classified as estuarine open water and are dominated by *Codium tenue* marcoalgal beds. Whitfield and Kok (1992) found that the juvenile mugilid species, *L. richardsonii* is most abundant in the lower and middle reaches of the Knysna Estuary. This was reflective of the results from this study with clear favouring of the marine bay and lagoon region of the system for juvenile Mugilidae. The adult Mugilidae showed a more widespread distribution with a hotspot in the upper estuary region. The findings from the seine netting sample set reflect the findings of Pollard (2017), in which Sparidae species showed a greater association with *Zostera capensis* vegetation than Mugilidae species. It is noticeable that all of the key MED species sampled Mugilidae species, *L. lithognathus* and *R. holubi* showed a dominance of juvenile specimens, this highlights the Knysna Estuary as a nursery habitat for the MED species. The E&M species *A. breviceps* and the SE species *H. capensis* showed a dominance of adult specimens, these fish species are using the estuarine habitats for a large majority of or whole life cycle, for these species the estuarine habitats are critical for adult specimens as opposed to acting as nursery habitats.

In the lagoon region of the estuary, a key hot spot was also identified for several important fishery species, including *P. saltatrix, R. holubi* and *S. salpa*. For *R. holubi,* a MED species that makes wide use of estuary systems (Whitfield 2019), it is not unexpected to find hotspots further upstream. While the other two species, *P. saltatrix* and *S. salpa,* which are MEO species, are known to recruit into the Knysna Estuary between September and December (Whitfield and Kok 1992), the upstream lagoon hotspot is unusual as they are usually associated with the lower reaches of estuaries with salinity playing a limiting factor for their upstream dispersal (Whitfield 2019). The marine influence of the Knysna Estuary, however, means that even at this point relatively far up the estuary, the salinity is well above 30 (Table 2.1, Chapter 2), which is well within the salinity range suitable for these species. When considering water temperature during the sampling period (see Table 2.1, Chapter 2), this region had the highest recorded temperatures, particularly during the summer sampling period, with much lower temperatures recorded in sites near the estuary mouth, most likely a result of cold ocean water upwelling, common during summer periods along the South African coastline (Day 1981). Thus, this hotspot may provide a thermal refuge for MED and MEO species from cold ocean water associated with summer upwelling in the system. A wide variety of habitats, including *Z. capensis, Z. capensis* mixed, *Caulerpa filiformis* mixed, and *Asparagopsis taxiformis* mixed habitats, are present along this bank (Figure 2.4, Chapter 2). This area has been shown to play an important role for MEO fishery species and therefore highlights its importance as a region for conservation.

In the lower estuary region, key hotspots were identified for the fishery species *L. lithognathus* and the dominant SE species *H. capensis.* The lower estuary hotspot for *L. lithognathus* is the second hotspot for this species. As with the marine bay hotspot, it is in an area of *Z. capensis* vegetation, confirming the Sparidae species associated with this vegetation type. The lower estuary hotspot for *H. capensis* is on the boundary between the system's lower and upper estuary regions. Being a SE species, it would be expected that the species would show distribution hotspots higher up the system. This region played host to several different habitats including *C. filiformis* mixed, *Z. capensis,* and *Z. capensis* mixed vegetation (Figure 2.4, Chapter 2).

One of the most notable unexpected hotspots identified was that of *Stolephorus holodon*. Being a MEO species, one would expect the abundance of *S. holodon* to be higher in the marine-dominated regions of the system. However, the results of the Kernel density distribution indicated a hotspot in the upper estuary region of the system. The catches of *S. holodon* were largely confined to a single sample, with 5 000 specimens of the total 5 052 specimens coming from a single site. All specimens of this species sampled also came from the winter sample period. This sizeable single catch led to a skewed overall MEO distribution, with the spatial distribution being representative of the single large catch of *S. holodon* rather than the guild's overall distribution. The hotspot is also unique as it is not focused on any productive vegetation type but is found on a mud bank classified as estuarine open water.

The hotspots identified in this study contribute significantly to our understanding of fish utilising the Knysna Estuary, being the first spatial assessment of littoral fish distribution across the entire system. Compared to previous long-term sampling efforts undergone by DFFE (2007-2012), species that didn't appear in this sampling effort were *Caranx herberi, Pomadys commersonnii, Chaetodon marleyi, Sardinops sagax, Gymnura natalensis, Engraulis japonicus, Galeichthys felis, Etrumeus whiteheadi, Myliobatis aquila, Aetomylaeus bovinus Lepomis macrochirus, Carcharias taurus* and *Cheimerius nafur*. The species from this list most notable in its absence from the seine net sampling effort is the MED spotted grunter, *Pomadasys commersonnii.* This species is a commonly targeted recreational fishery species within the Knysna Estuary (Whitfield et al. 2021), generally associated with shallow mud banks where these fish feed on invertebrates such as mud prawns *Upogebia africana* (Whitfield 2019). Several *P. commersonnii* were seen during the sampling efforts in clear water while moving between sites. However, none were present in the sample nets.

Pomadasys commersonnii has previously been sampled in the Knysna Estuary using seine nets (Grindley 1985, DFFE unpublished data, James and Harrison 2008) indicating that seine netting can be a suitable sampling method for the species. Butler et al. (2022) also published a report documenting a spawning aggregation of *P. commersonnii* in February 2017, which took place over a shallow bank along Leisure Island in the marine bay region of the Knysna Estuary. Juvenile *P. commersonnii* have been found to have a thermal preference for 24-25 °C (Deacon and Hecht 1995, 1996), which was seldom reached during the sampling effort, with only three sites having recorded temperatures of over 24 °C. The lack *of P. commersonnii* is likely to be attributed to the low temperatures along with low turbidity, which allows the fish to be more aware of the seine net. This species not being present in the sample set highlights the need for continuous sampling in the Knysna Estuary system in order to better understand this essential fishery species' use of the estuary. Understanding spatial usage of the estuary by fishery species is vital for informing spatially targeted conservation measures within the estuary.

While catches from this seine net sampling provide an important overview of littoral fish assemblages in the Knysna Estuary, to properly understand spatial distributions of the overall fish assemblages, it is important to also consider demersal fish assemblages. Incorporating both littoral and demersal habitats is critical for effective conservation planning and the protection of the overall fish diversity. As a result, Chapter 4 will provide a spatial assessment of the demersal fish assemblages in the Knysna Estuary.

CHAPTER FOUR

SPATIAL ANALYSIS OF THE DEMERSAL FISH ASSEMBLAGE WITHIN THE KNYSNA ESTUARY

4.1 Introduction

Estuarine fish assemblage research in South Africa has been primarily dominated by seine netting sampling (Whitfield 2019), which allows for rapid, cost-effective sampling of littoral fish assemblages (Percer et al. 1990). This dominance of littoral sampling in estuaries has meant that, for the most part, demersal fish assemblages in estuaries have been under-sampled in South African estuaries. The complex nature of habitats in a large marine-dominated system such as the Knysna Estuary provides a wide variety of demersal habitats for fish species. Incorporating demersal habitats and associated fish species within fish assemblage research is essential to understand patterns and processes fully.

Demersal estuarine fish live on or near the substratum of an estuary; these fish species can be either benthic and rest on the substratum, or benthopelagic, which float in the water column above the substratum (Carl 2009). Globally, various trawl nets have been used to effectively sample demersal fish assemblages, including beam (Pomfret et al. 1988, Marshall and Elliot 1998, Dolbeth et al. 2008, Selleslagh and Amara 2008) and otter trawls (Petrik and Levin 1999, Johnson et al. 2008, Bishop et al. 2015). The beam trawl has been shown to be an effective sampling method for a variety of early juvenile demersal fish (Hemmingways and Elliot 2002, Harrison et al. 2017). It has the added advantage of a constant mouth-opening size, thereby reducing potential sample biases (Kuipers et al. 1992).

In South Africa, only work done by Nodo et al. (2017), Nodo et al. (2018), and Nodo (2022) have made use of a shoeless beam trawl in estuarine habitats with Harrison and Whitfield (1995), making use of both beam and otter trawls and Richardson et al. (2006) making use of only otter trawls. Work done by Nodo et al. (2017, 2018) looked at the effect of flooding on demersal fish assemblages in the Great Fish and Kariega estuaries, respectively, whilst beam trawls were used to assess the roles of benthic habitats in estuaries and nearshore marine areas in Algoa Bay (Nodo et al. 2022). Harrison and Whitfield (1995) used beam and otter trawls to sample the fish assemblages in three temporary open/closed estuaries in Kwazulu Natal, the Mhlanga, Damba and Zotsha estuaries. Richardson et al. (2006) made use of an otter trawl to investigate the effect of water temperature, salinity, and turbidity on the distribution of demersal fish in the Kariega Estuary in the Eastern Cape.

The lower region of the Knysna Estuary is classified as an estuarine embayment with similar physiochemical characteristics to the surrounding marine habitat within a sheltered region (Largier et al., 2000; Whitfield 2019) due to the wide, deep mouth and strong marine influence. This has led to observations of numerous fish species, particularly marine stragglers, which are not typically associated with estuarine habitats (Whitfield and Kok 1992, James and Harrison 2008). A number of important demersal linefish species have also been noted in the estuary both through previous research and anecdotal observations; three notable species include white musselcracker (*Sparodon durbanensis*) (Grindley 1985), santer (*Cheimerius nufar*) (Unpublished DFFE sample set), and red roman (*Chrysoblephus laticeps*) (Smith pers.comm). Although marine species have been noted in the Knysna Estuary, there has been little work done on understanding why these fish have been present in the system and what life stages of these fish are utilizing the estuarine habitats. The marine dominance and demersal habitats available to fish in the Knysna Estuary facilitate the need for a study that specifically targets this previously ignored group of fish.

The aim of this chapter was to spatially assess the demersal fish assemblage within the Knysna Estuary to identify essential fish habitats to inform effective conservation planning. The specific objectives of this study were to (i) describe the demersal fish assemblage in the Knysna Estuary and (ii) use optimised hotspot and Kernel density analyses to identify key density hotspots of demersal fish species by estuarine classification, dominant species, and life stages in the Knysna Estuary.

4.2 Methodology

4.2.1 Fish community sampling

The demersal fish assemblage in deeper channels was sampled using a conical shoeless beam trawl net designed by Harrison et al. (2017). Constructed from 14-mm knotless nylon mesh with dimensions of 1.5 m wide × 0.5 m high × 3 m body length, tapering to 0.5 m width at the codend (Harrison et al. 2017, Nodo et al. 2017, Nodo et al. 2018). The codend was 1 m long and constructed of 6.5 mm knotless nylon mesh. The net was weighted with a 55 mm short link chain with two 0.5 m lengths of 10 mm chain attached to the footrope to ensure bottom contact. The net was towed at a distance of 20 m behind the boat on shallow sites (<5 m) and 40 m behind the boat on sites with a depth of over 5 m. Towing time was standardised at five minutes except for four sites where space or dense vegetation limited trawls to three minutes; these were the site in the Thesen Island canals. The net was pulled at an approximate speed of two knots (methods used for beam trawl sampling by Nodo et al. (2017 and 2018)), although slight differences in speed due to depth or vegetation densities were noted.

All fish sampled were identified to species level and total length measured (mm TL). At sites with large catches of a single species, 80 representatives of that species were measured, and thereafter individuals were counted and released.

Sampling dates were chosen to coincide with the peak recruitment period identified for the majority of species (Appendix 2). Localised flooding in the Garden Route area caused a delay in the initial sampling from October 2021 to November 2021. The second sampling period was undertaken in March 2022. On both occasions, sampling was completed over a two-day period.

4.2.2 Sample sites

Beam trawling took place at 25 sites (Figure 4.1) within the general vicinity of the seine net sampling sites (Figure 3.1, Chapter 3), dependent on habitat characteristics. Beam trawling is not successful within dense macrophyte beds, and densely vegetated sites were therefore avoided.

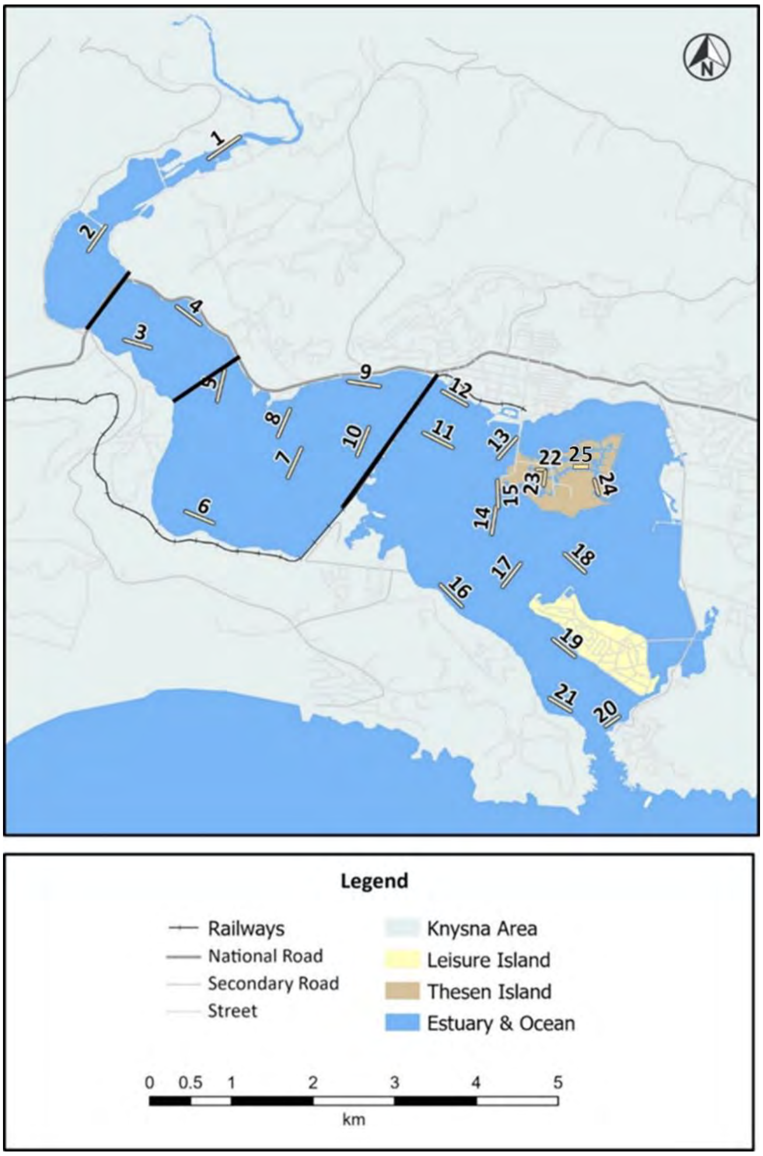


Figure 4.1: Map of the Knysna Estuary illustrating the 25 beam trawl sample sites throughout the estuary with estuarine regions indicated. Each site was situated in the selected seine net sites (see Chapter 3) adjacent channel where possible.

4.2.3 Data analysis

4.2.3.1 Spatial distribution of estuarine guilds

All species were classified into the estuarine use functional guilds (Whitfield et al. 2019) (Table 2, Chapter 2). The CPUE for the species table calculation was done using the mean densities of catches over the two sampling periods; the average distance per full beam trawl was calculated with shorter beam trawls distance being calculated. The density of observation was calculated (fish per 1000 m^2) using the formula as per Harrison et al. (2017):

CPUE = total number of individuals at each site ÷ area trawled (in m) × 1000

Due to a relatively low variation in catch abundance, the mean density data for each site of the two sampling periods were square root transformed using PRIMER statistical package version 7.0.13 prior to GIS spatial analysis in ArcGIS Pro v 2.8.1. Using the square root transformed average catch data, spatial distribution maps of each estuarine guild were calculated using the Kernel density tool in ArcGIS Pro.

Kernel density, calculation was undertaken for each estuarine guild using the transformed catch numbers for the entire sampling period; being a density tool, it was not necessary to use CPUE as the Kernel density input as the output of the Kernel density tool is observation/km^2. A 10 m resolution raster was used, based on a search radius of 5 km and 12 nearest neighbours. Five kilometres was utilised for the search radius as it approximated half the Euclidian length of the estuary.

4.2.3.2 Spatial distribution of dominant species by life stage

The species with the highest catch from each estuarine guild were selected in order to compare life stage distribution patterns. These species were as follows: estuarine and marine (E&M) *Clinus superciliosus*, marine estuarine opportunist (MEO) *Spondyliosoma emarginatum*, marine estuarine dependent (MED) *Heteromycteris capensis* and solely estuarine (SE) *Clinus spatulatus*. The marine straggler (MS) and estuarine and freshwater (E&F) guilds were excluded from this analysis due to the low catches of species in these guilds, with five and 15 total samples, respectively.

The average catch over the two sampling periods for each life stage was calculated at each site and used to develop Kernel density and distribution maps. Using the same scale as total catches, the Kernel density of different life stages was plotted, enabling life stages to be represented as a proportion of the total catch. As with the estuarine guild kernel densities, a 10 m resolution raster was used based on a search radius of 5 km and 12 nearest neighbours.

4.3 Results

4.3.1 Species composition and abundance

The total catch over two sampling periods comprised 2894 individuals from 24 species and 13 families. Seven MEO, five E&M, four MED, three SE and one E&F species were caught. The M&E species *C. superciliosus* was the most dominant species caught, contributing with an overall CPUE of 57.7 individuals/km^2 (n=1446, 50%). Adult *C. superciliosus* dominated the catch (CPUE = 36.4 individuals/km^2), followed by juveniles (CPUE = 21.2 individuals/km^2). Other species dominating the catch were SE *Clinus spatulatus,* with an overall CPUE of 12.0 individuals/km^2 (n=241, 8 %), dominated by juveniles (CPUE = 10.9 individuals/km^2) followed by adults (CPUE = 1.1 individuals/km^2). The MED *Heteromycteris capensis* had an overall CPUE of 13.4 individuals/km^2 (n=364, 13%), dominated by juveniles (CPUE = 12.4 individuals/km^2) followed by adults (CPUE = 0.7 individuals/km^2) and settlement stage (CPUE = 0.3 individuals/km^2). The MEO species S. *emarginatum* had an overall CPUE of 15.8 individuals/km^2 (n=306, 11 %), dominated by juveniles (CPUE = 13.7 individuals/km^2) followed by settlement stage (CPUE = 2.1 individuals/km^2). (Table 4.1).

Table 4.1: Species composition of combined beam trawl sampling in the Knysna Estuary during November 2021 and March 2022. Estuarine guild classification as per Whitfield (year). The total number of individuals and proportion of the total catch, catch per unit effort (density of fish per km), fish length (mean and range TL mm) life stage CPUE (individuals/km^2) and composition (% of total catch) and estuarine region of species caught (FS=full system, MB=marine bay, L=lagoon, LE=lower estuary, UE=upper estuary) is provided.

Family	Species	Number of individuals (% of total catch)	CPUE	Mean length (range) mm	Settlement CPUE (% species catch)	Juveniles CPUE (% species catch)	Adult CPUE (% species catch)	Estuarine region
Estuarine and freshwater								
Gobiidae	*Glossogobius callidus*/ River Goby	15 (0.5)	0.5	24 (17-70)		0.5 (93.3)	<0.1 (6.7)	FS
Solely estuarine								
Clinidae	*Clinus spatulatus*	241 (8.3)	12	52 (27-90)		10.9 (88.0)	1.1 (12.0)	LE – MB
Clupeidae	*Gilchristella aestuaria*/ Estuarine roundherring	7 (0.2)	0.3	25 (18-39)		0.2 (85.7)	<0.1 (14.3)	UE
Syngnathidae	*Hippocampus capensis*/ Knysna seahorse	5 (0.2)	0.2	43 (23-54)		0.2 (100.0)		MB
Marine and estuarine								
Clinidae	*Clinus superciliosus*/ Super klipvis	1 446 (50)	57.7	55 (14-105)		21.2 (35.6)	36.4 (64.4)	LE-MB
Gobiidae	*Caffrogobius gilchristi/* Prison goby	10 (0.3)	0.4	53 (25-110)		0.2 (60.0)	0.1 (40.0)	FS
Gobiidae	*Caffrogobius nudiceps*/ Barehead goby	137 (4.7)	5.3	54 (29-105)		2.0 (37.2)	3.3 (62.8)	LE-MB
Gobiidae	*Psammogobius knysnaensis*/ Knysna sandgoby	69 (2.4)	2.6	42 (24-74)		0.1 (4.4)	2.5 (95.7)	FS
Syngnathidae	*Syngnathus temmincki*/ Longsnout pipefish	68 (2.3)	2.6	137 (67-200)		0.6 (23.5)	2.0 (76.5)	LE-MB
Marine-estuarine dependant								
Sciaenidae	*Argyrosomus japonicus*/ Dusky kob	1 (0.03)	<0.1	15	<0.1 (100)			UE
Soleidae	*Heteromycteris capensis*/ Cape sole	364 (12.6)	13.4	56 (11-85)	0.3 (2.2)	12.4 (92.9)	0.7 (5.0)	LE-MB
Soleidae	*Solea turbynei*/ Blackhand sole	25 (0.9)	1.1	56 (17-85)	<0.1 (4)	0.3 (28.0)	0.8 (68.0)	FS
Sparidae	*Rhabdosargus holubi*/ Cape Stumpnose	22 (0.8)	0.8	64 (16-138)	<0.1 (4.6)	0.8 (95.5)		LE-MB
Marine-estuarine opportunists								

Haemulidae	*Pomadasys olivaceum*/ Piggy	13 (0.5)	0.5	39 (30-83)	0.2 (46.2)	0.3 (53.9)		LE-MB
Platycephalidae	*Platycephalus indicus*/ Bartail flathead	1 (<0.1)	<0.1	432		<0.1 (100.0)		L
Sparidae	*Diplodus capensis*/ Blacktail	12 (0.4)	0.4	72 (25-99)	<0.1 (8.3)	0.4 (91.7)		L&MB
Sparidae	*Rhabdosargus globiceps*/ White stumpnose	46 (1.6)	1.7	24 (16-83)	1.3 (80.4)	0.3 (19.6)		FS
Sparidae	*Sarpa salpa*/ Strepie	87 (3.0)	3.2	49 (32-77)		3.2 (100)		MB
Sparidae	*Spondyliosoma emarginatum*/ Steenjie	306 (10.6)	15.8	51 (20-94)	2.1 (14.7)	13.7 (85.3)		LE-MB
Tetraodontidae	*Geneion honckenii*/ Evileye blaasop	9 (0.3)	0.3	63 (16-152)	0.1 (22.2)	0.2 (66.7)	<0.1 (11.1)	FS
Cynoglossidae	*Cynoglossus capensis*/ Sand toungefish	4 (0.1)	0.1	64 (35-90)		0.1 (100.0)		Marine bay
Marine stragglers								
Gobiidae	*Apletodon pellegrini*/ Chubby klingfish	2 (<0.1)	0.1	31 (27-35)		0.1 (100.0)		L&MB
Priacanthidae	*Priacanthus harmur*/ Lunar-tailed bigeye	1 (<0.1)	<0.1	60		<0.1 (100.0)		MB
Triglidae	*Chelidonichthys capensis*/ Cape Gurnard	3 (0.1)	0.1	66 (58-78)		0.1 (100.0)		MB

The catches for each region of the estuary were variable, with MEO species showing a decreased proportion of the total catch moving from being dominant in the upper estuary towards the making up a small proportion of the total catch in the marine bay (Figure 4.2). M&E species showed an increased proportion moving from a small proportion of the upper estuary catch towards dominance in the marine bay. Marine estuarine dependent (MED) species showed relatively constant proportion of the catch, MS species made up a small proportion in every estuary region and E&F species were only present in the upper estuary region.

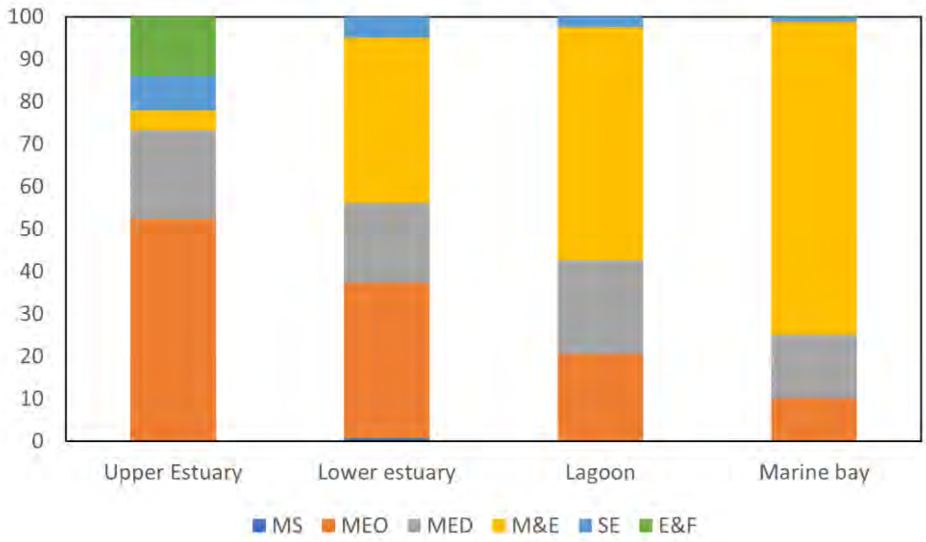


Figure 4.2: Percentage composition of estuarine guilds (MS = marine stragglers, MEO = marine-estuarine opportunists, MED = marine-estuarine dependents, M&E = marine and estuarine, SE = solely estuarine, E&F = estuarine and freshwater) to the total catch-per-unit effort sampled in the different regions of the Knysna Estuary during November 2021 and March 2022.

Overall, the catch of E&F, MED, MEO, and MS guilds were dominated by juvenile life stages. Adult life stages dominated the SE and M&E guilds. Settlement stage individuals were only present in the MED and MEO guilds making up a small proportion of the catch for both these guilds (Figure 4.3).

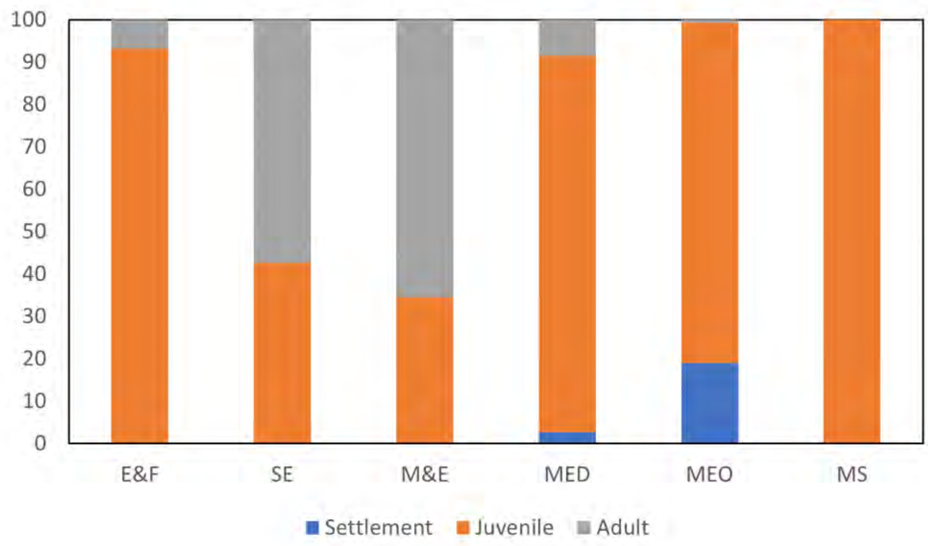


Figure 4.3: Percentage composition of life stage per guild (E&F = estuarine and freshwater, SE = solely estuarine, M&E = marine and estuarine, MED = marine-estuarine dependents, MEO = marine-estuarine opportunists, MS = marine stragglers) to the total catch-per-unit-effort sampled in the Knysna Estuary during November 2021 and March 2022.

4.3.2 Spatial distribution of functional estuarine guilds

The highest catch of all the functional estuarine guilds was observed in the marine bay section, mainly around Thesen Island. The E&F guild exhibited a hotspot in the upper estuary region of the system, with a minor hotspot in the marine bay region. The low catch number for this guild means the hotspot is not reflective of high catches. The SE guild was widely represented throughout the marine bay region of the system, with a hotspot at the head of Thesen Island. There was a secondary hotspot that extended through the top end of the lagoon and throughout the lower estuary region of the system. The M&E guild were represented throughout the system but with a clear hotspot in the main channel of the marine bay region at the head of Thesen Island. This guild showed a secondary hotspot covering much of the lagoon region of the system (Figure 4.4).

The MED guild was widely distributed throughout the system, with a widespread hotspot throughout much of the marine bay region extending from the middle of Leisure Island to the head of Thesen Island. The MEO guild was widely distributed throughout the system and exhibited a significant hotspot in the marine bay region at the start of the Ashmead channel between Leisure Island and Thesen Island. There was high catch density throughout much of the marine bay region of the system. The MS guild showed a hotspot in the marine bay region at the start of the Ashmead Channel between Leisure Island and Thesen Island. There was a secondary hotspot in the lower estuary region of the system. The low catch numbers mean that these hotspots are not reflective of an area with high density (Figure 4.4).

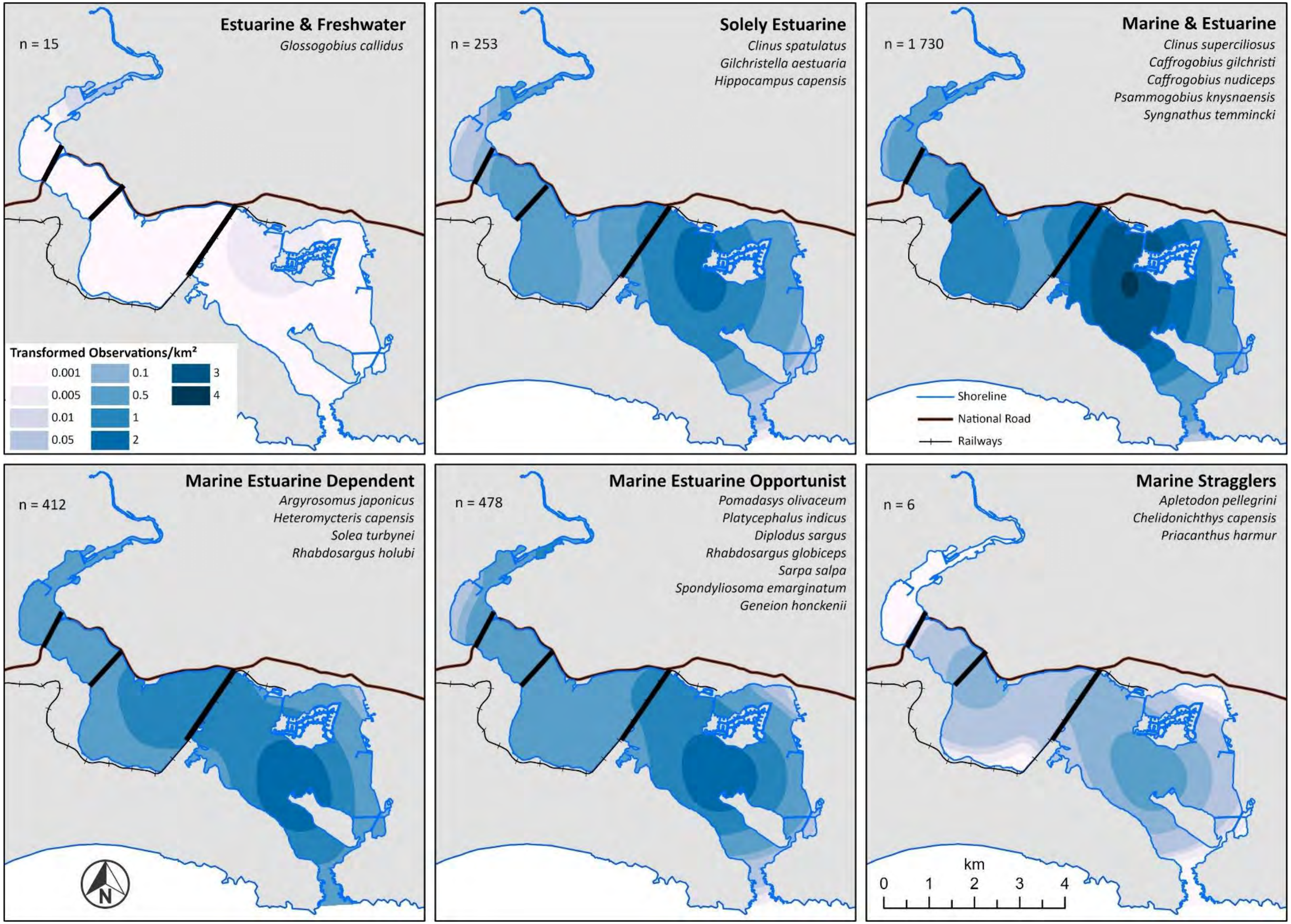


Figure 4.4: Kernel density spatial distribution maps of the average catch over two sampling trips (November 2021 and March 2022) for beam trawl catches within the Knysna Estuary categorised by their estuarine guilds (as defined by Whitfield 2019), with total catches indicated (n). Data were square root transformed prior to GIS analysis

4.3.3 Life stage distribution of dominant species

The overall density hotspot for *C. supercilious* was in the estuary's marine bay region, near the head of Thesen Island and Leisure Island, with an estimated CPUE of 137.3 individuals/km^2. Both juvenile and adult *C. superciliosus* were present in this density hotspot, with CPUE of 56.4 individuals/km^2 and 80.9 individuals/km^{2,} respectively (Figure 4.5).

The overall density hotspot for *Clinus spatulatus* was in the marine bay region, near the entrance to the Thesen Island Canals, with an estimated CPUE of 34.2 individuals/km^2. The juveniles were primarily responsible for this hotspot, with an estimated CPUE of 31.8 individuals/km^2, while the adults were much less prevalent, with a slight hotpot in the marine bay region at the head of Thesen Island, with an estimated CPUE of 4.1 individuals/km^2 (Figure 4.6).

The overall density hotspot for *Heteromycteris capensis* was in the marine bay region of the estuary, at the head of Leisure Island, with an estimated CPUE of 70.3 individuals/km^2 dominated by juvenile specimens, with a CPUE of 64.8 individuals/km^2 for this area. Adult *H. capensis* showed a hotspot in the same area, with an estimated CPUE of 4.2 individuals/km^2. The settlement stage individuals' catches were too low to properly reflect an accurate hotspot for this life stage (Figure 4.7).

The overall density hotspot for *Spondyliosoma emarginatum* was in the marine bay region between Thesen Island and Leisure Island, with an estimated CPUE of 21.1 individuals/km^2; this hotspot was dominated by juvenile specimens with an estimated CPUE of 16.2 individuals/km^2 in this area and settlement stage with an estimated CPUE of 4.9 individuals/km^2. *Spondyliosoma emarginatum* exhibits a secondary overall and juvenile hotspot in the lagoon region of the estuary, with an estimated CPUE of 49.1 individuals/km^2 and a juvenile CPUE of 47.3 individuals/km^2 (Figure 4.8).

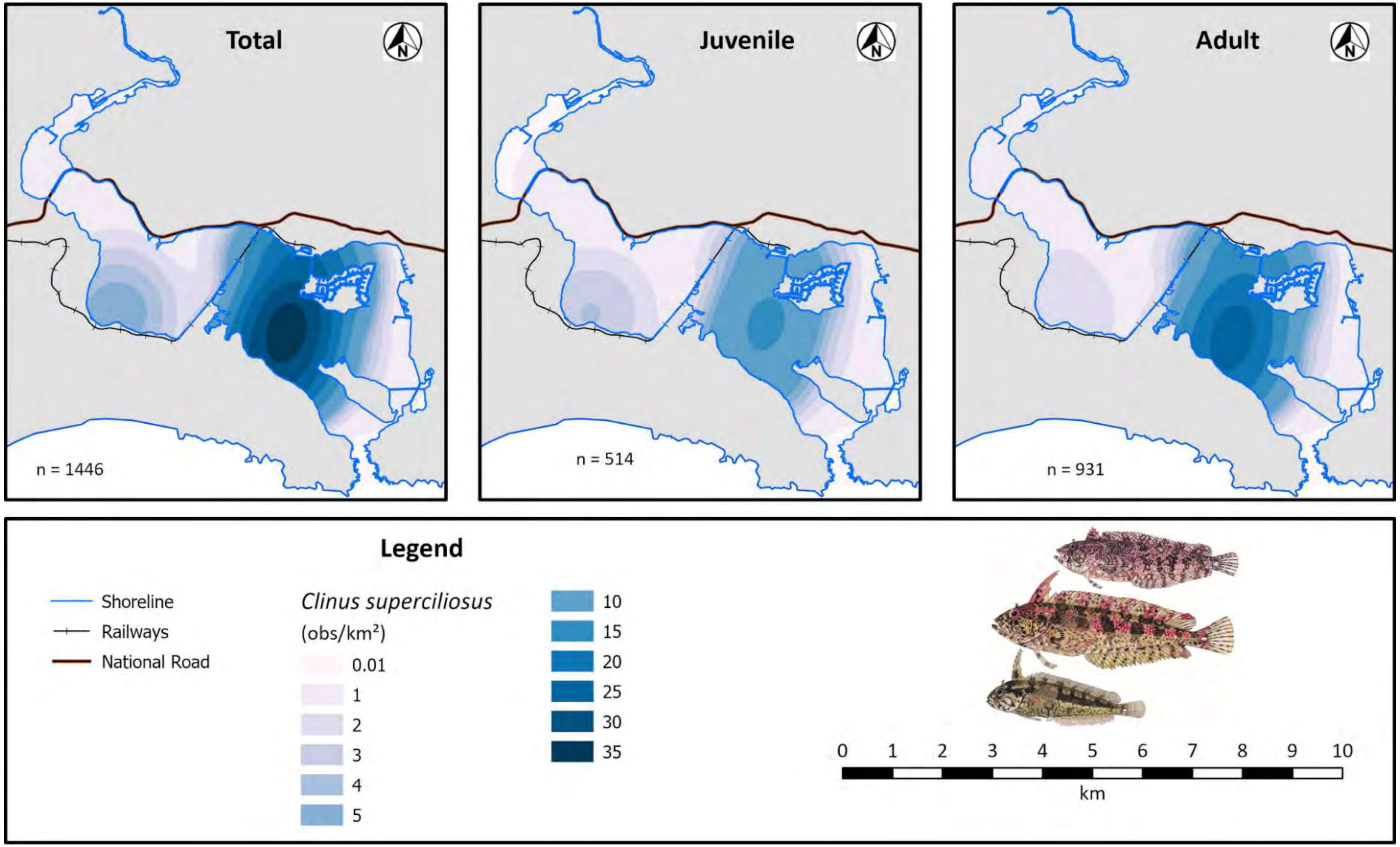


Figure 4.5: Kernel density spatial distribution maps of the average catch over two sampling trips (November 2021 and March 2020) for the dominant estuarine and marine (E&M) species sampled in the Knysna Estuary, *Clinus superciliosus*. Densities represent catch-per-unit-effort, including total, juvenile, and adult specimens sampled using beam trawl. n = catch numbers.

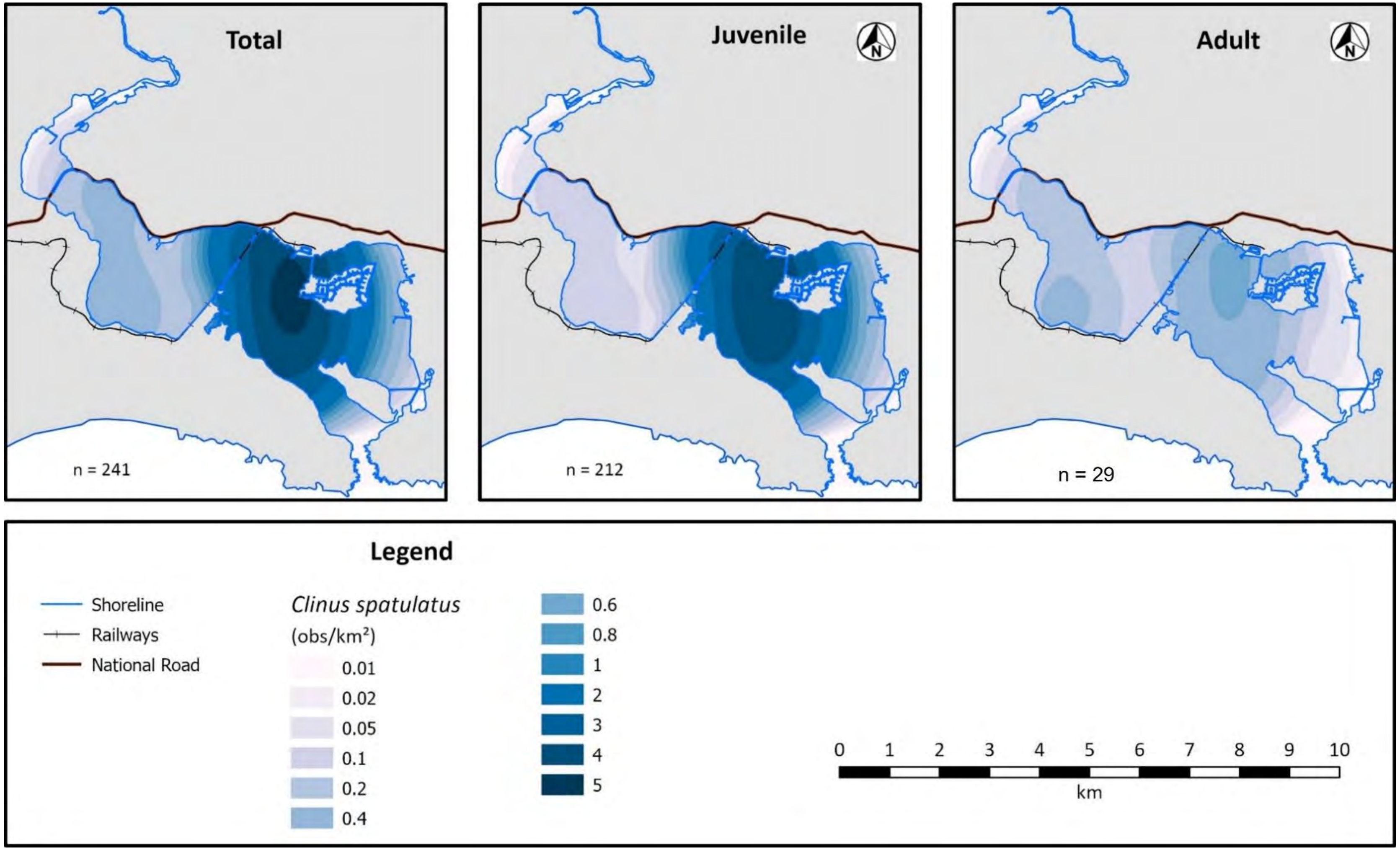


Figure 4.6: Kernel density spatial distribution maps of the average catch over two sampling trips (November 2021 and March 2022) for the dominant solely estuarine (SE) species sampled in the Knysna Estuary, *Clinus spatulatus*, including total, juvenile, and adult specimens sampled using beam trawl. n = catch numbers.

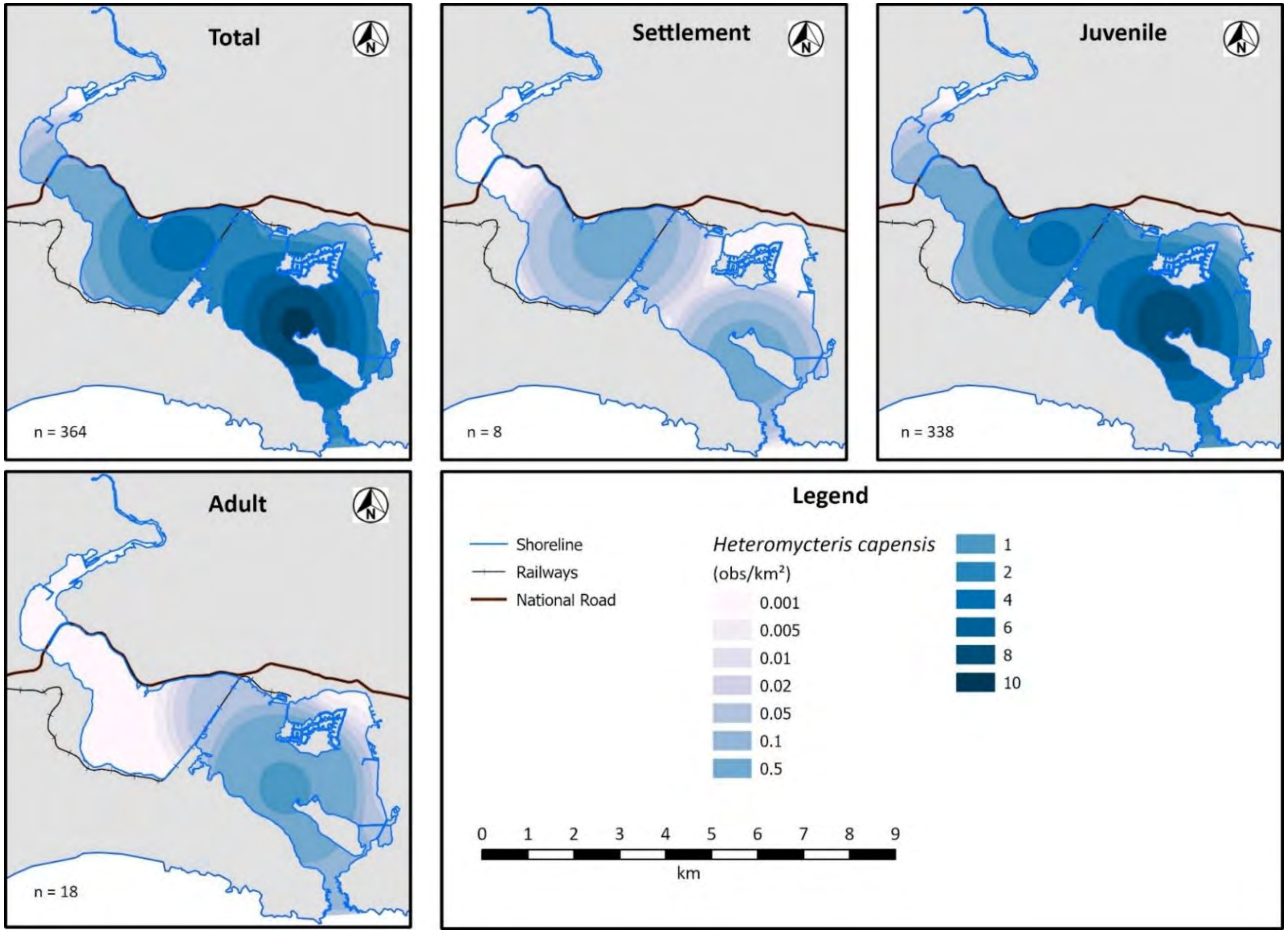


Figure 4.7: Kernel density spatial distribution maps of the average catch over two sampling trips (November 2021 and March 2022) for the dominant marine-estuarine dependent (MED) species sampled in the Knysna Estuary *Heteromycteris capensis,* including total, settlement, juvenile, and adult specimens sampled using beam trawl. n = catch numbers.

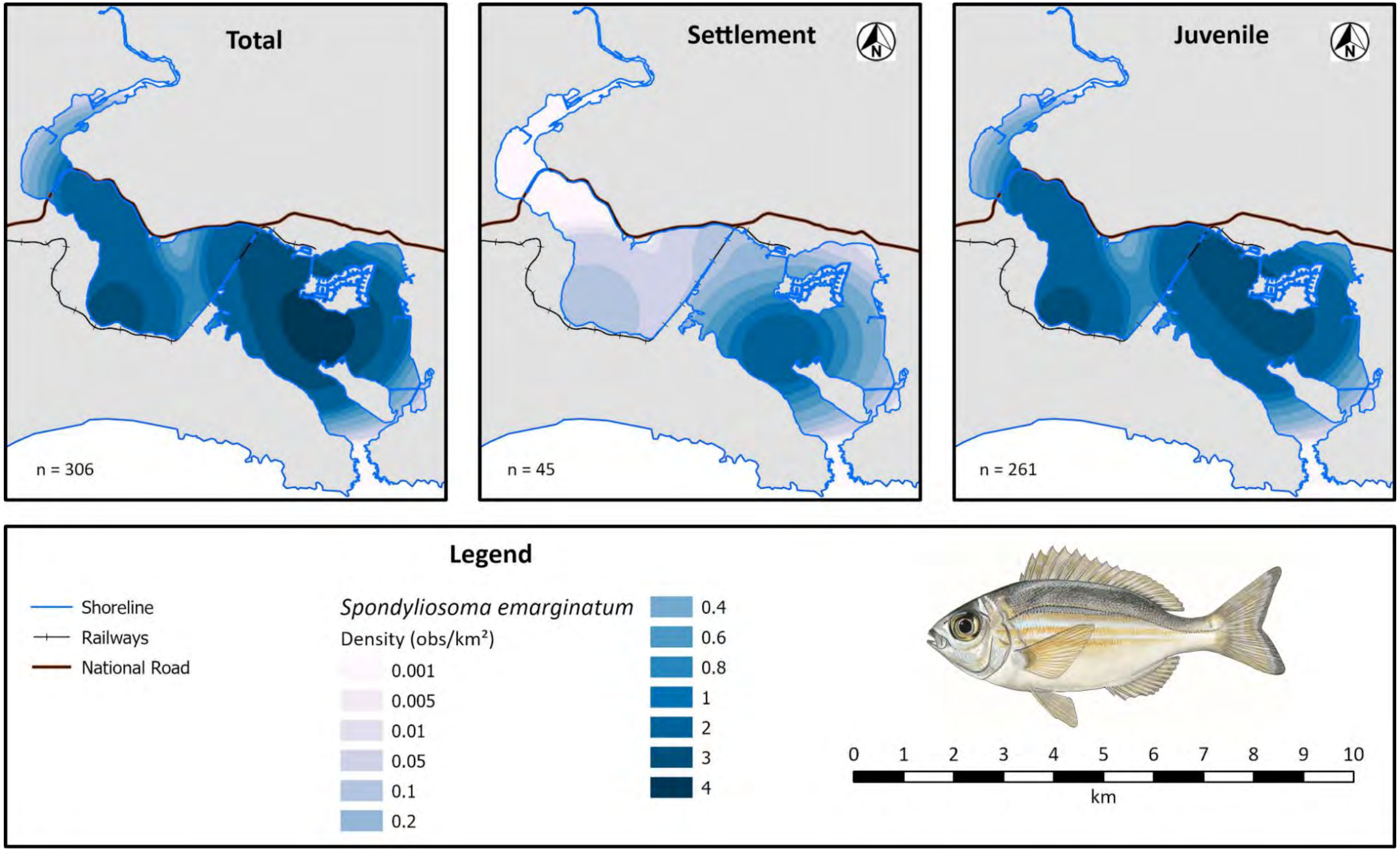


Figure 4.8: Kernel density spatial distribution maps of the average catch over two sampling trips (November 2021 and March 2022) for the dominant marine-estuarine opportunist (MEO) species sampled in the Knysna Estuary *Spondyliosoma emarginatum* including total, settlement and juvenile specimens sampled using beam trawl. n = catch numbers.

4.4 Discussion

The results presented here indicate key hotspot areas for different guilds, dominant species, and life stages of demersal fish species within the Knysna Estuary. Beam trawl catches were dominated by marine and estuarine species, primarily due to a single species, *Clinus superciliosus*, which made up 50 % of the total catch. All of the estuarine guilds showed high numbers of juvenile specimens, with only the SE and M&E guilds showing adult dominance and a very low overall representation of settlement stage samples. The marine bay area showed the highest density hotspots for the dominant species (*C. superciliosus, C. spatulatus, H. capensis*, and *S. emarginatum*) identified for all life stages.

As with previous demersal sampling in South African estuaries by Harrison and Whitfield (1995), Richardson et al. (2006), Bailey and James (2013), Nodo et al. (2017), and Nodo et al. (2018), Gobidae and Soliidae were well represented making up 7.4 % and 13.5 % of the total catch respectively. However, in contrast to both Richardson (2006) and Bailey and James (2013), where Clinidae made up less than one percent of the total catch, Clinidae was the most dominant family comprising 58.3 % of the total catch in this study, both Clindae species sampled in this effort show temperate distribution which means the Eastern Cape estuaries fall at the edge of these species distribution, likely playing a role in the lower abundance when compared with the Knysna Estuary. Both earlier studies were conducted in the Kariega Estuary in the Eastern Cape of South Africa, which although also marine dominated is a narrow, shallow, and linear estuary with a band of *Z. capensis* vegetation along much of the bank (Watling and Watling 1983, Grange 1992). The Knysna Estuary, however, is drastically different with its deep and complex nature and large complex *Z. capensis* beds dominating much of the system's intertidal and shallow subtidal areas as opposed to linear, narrow *Z. capensis* beds associated with the Kariega Estuary.

Clinus supercilious, a demersal Clinidae species is often associated with *Zostera capensis* (Whitfield 2019), dominated beam trawl catches, while *C. spatulatus* was well represented in catches, being the SE species with the largest catch. The sites with high catches of both these species were located in the middle area of the marine bay above Thesen and Leisure Island, an area in which the shallow intertidal banks are dominated by *Z. capensis*, the deeper channels adjacent to these vegetated beds where beam trawls where undertaken is, however, largely unvegetated with sparse *Z. capensis*. The area around the head of Thesen Island, however, includes a large amount of "rocky" habitat in the river channel, which greatly increases the habitat complexity of this region of the system. As the beam trawl sampling was not possible in the dense *Z. capensis* beds, these large catches of Clinidae species when compared with seine net sampling (Chapter 3) which was undergone in the dense *Z. capensis* vegetation, indicates that these fish are making use of the less dense vegetation found on the edges of *Z. capensis* beds.

Clinus superciliosus is associated with rocky regions and areas of vegetation in the lower reaches of estuaries (Whitfield 2019). This is reflective of the sampling results from this research with the hotspot near the head of Thesen Island, an area with widespread *Z. capensis* beds and then rocky structure used to build up the Thesen Island canals. *Clinus superciliosus* also showed a higher proportion of adults to juveniles indicating that this species is making use of the system throughout its life cycle as opposed to using the system as a nursery zone. *Clinus superciliosus* is recorded breeding year-round, with peak spawning occurring during winter and spring (Veith 1979). Only juvenile and adult representatives of *C. superciliosus* and *C. spatulatus* were present in the beam trawl catches potentially due to the short pelagic larval stage of Clinidae and their rare contribution in near-shore larval sampling (Hollerman et al. 2012). The beam trawl has been shown to be an effective sampling method for larval stage fish (Nodo et al. 2022) however it is still more effective for sampling larger specimens and is not specifically targeting larval specimens. In order to best understand larval fish usage of the Knysna Estuary specific larval sampling methods would be required.

The MED species with the largest catch, *Heteromycteris capensis*, is a species associated with bare sand, being flat and perfectly adapted to camouflage on sandy bottom areas (Cyrus and Martin 1991). Sediment characteristics appear to significantly influence this species' distribution longitudinally throughout estuarine systems (Whitfield 2019). These fish are not associated with the muddy reaches of estuarine systems but appear to be found in the lower reaches of estuaries where sandy bottoms are the dominant demersal habitat (Richardson et al. 2006; Bailey and James 2013). The main hotspot for this species in Knysna is representative of this pattern with a large sandbank with minimal vegetation around Leisure Island being identified as a primary hotspot area. The secondary hotspot for this species above the railway bridge is sandy with little vegetation. Juveniles of this species appear to make wide use of the lower reach of the system, but there is a clear association with sparsely vegetated or bare sites.

A species commonly sampled in the beam trawl was *S. emarginatum*, a marine estuarine opportunist (MEO) species. Although this species is not often associated with littoral estuarine habitats (Fairhurst et al. 2010), settlement and young-of-the-year juveniles were associated with demersal channel habitats in the permanently open Sundays and Swartkops estuaries in Algoa Bay (Nodo et al. 2022). Larger individuals have also been noted in two estuarine systems near Knysna Estuary, the Swartvlei Estuary, and the Keurbooms Estuary. Research in the Keurbooms Estuary using baited remote underwater video cameras has shown *S. emarginatum* making use of the estuary (Elson via pers.comm), while annual seine net sampling in the Swartvlei Estuary has also produced sporadic catches in low abundance (Smith via pers.comm). *Spondyliosoma emarginatum* has previously been sampled in Knysna Estuary, with a large number of juveniles being recorded in the lower reaches during a summer seine net sampling effort (Whitfield and Kok 1992). Similar to other MEO sparid species sampled in the beam trawl (*Sarpa salpa, Diplodus capensis*, and *Rhabdosargus globiceps*), no adult specimens of *S. emarginatum* were present. Results from this study indicate that *S. emarginatum* appears to make relatively wide use of the Knysna Estuary as a nursery area with juveniles present from the marine bay region up to the lower estuary region of the system.

This marine dominance in the system has been noted in previous studies (Whitfield and Kok 1992, James and Harrison 2008). Unique marine species sampled in the beam trawl during this study included *Apletodon pellegrini, Priacanthus harmur*, and *Chelidonichthys capensis*. It would be expected that these species would mostly be present in the estuary's lower, more marine-dominated reaches. Although only six individuals within the MS guild were sampled, all bar one was caught in the marine bay section. There was one specimen of *A. pellegrini* which was sampled higher up the estuary at site BT5 in the lower estuary region of the system.

The beam trawl has been shown to be an effective method of sampling demersal fish species in Eastern Cape estuaries as well as the nearshore areas of Algoa Bay (Nodo et al. 2017; 2018; 2022) and now the Knysna Estuary. This project made use of 25 beam trawls, which were distributed evenly throughout the estuary in order to account for the complexity of the habitat network present in the system. Demersal sampling efforts using beam or otter trawls in more linear, shallow estuary systems have tended to produce catches that are relatively representative of overall fish demersal distributions (Richardson et al. 2006). All known taxa apart from Mugilidae have been successfully sampled using demersal trawls (Richarson et al. 2006).

The notable catches of demersal fish species using beam trawl sampling in the Knysna Estuary highlights the need for a focus on the demersal fish, which are usually not adequately considered when using generally accepted littoral estuarine sampling methods such as seine netting. Demersal fish assemblages form an important component of the overall estuarine fish assemblages, which has been ignored when considering overall fish assemblages and their conservation in the Knysna Estuary. The beam trawl sampling acts as an efficient supplementary sampling method to seine net sampling when considering the overall fish assemblages in the Knysna Estuary. Considering these demersal fish species is of vital importance when considering spatial planning for conservation in estuarine systems.

CHAPTER FIVE

GENERAL DISCUSSION

The Knysna Estuary has been identified as the estuary with the highest conversation priority in South Africa; it is the only "estuarine bay" system in the warm-temperate region of South Africa and is the largest estuary system on the southern coastline of South Africa (Whitfield 2019). This study highlighted the importance of the Knysna Estuary for fish, with a total of 47 species recorded. The Knysna Estuary is particularly important for marine species, with both the littoral and demersal fish assemblage dominated, in terms of richness, by marine-estuarine opportunist (MEO) species. The spatial analysis highlighted the importance of the "marine bay" region of the system, with the majority of functional estuarine guilds and key ecological and fishery species abundant in this region. This was especially obvious for the demersal sampled beam trawl species, with every functional guild showing hotspots in the marine bay region of the system (Figure 4.4, Chapter 4). While all functional estuarine guilds (except for the E&F guild, which exhibited a hotspot in the Lagoon region) of the littoral fish assemblage sampled with the seine net also exhibited marine bay hotspots, a more diverse hotspot distribution was noted (Figure 3.4, Chapter 3). The marine dominance of the Knysna Estuary was evident in the sampled catches, with 98.5% of seine net catches, and 90.8% of beam trawl catches comprising marine taxa. This is notably higher than the contribution of marine taxa (between 68 and 75%) associated with South African estuaries, as stated by Whitfield (2005). Protecting the critical *Zostera capensis* habitat in the marine bay region of the system, which is under huge pressure due to eutrophication in the Ashmead Channel, is a priority due to its importance as habitat for fish species and overall estuary nursery function. Another important density hotspot for fishery species was identified in a small section of the lagoon region of the estuary; this finding is significant as this area is currently classified by SANParks as a low-sensitivity habitat affording no protection to the fish species making use of this area.

5.1 Sensitivity mapping

The current SANParks sensitivity zones (GRNP 2020) in place within the Knysna Estuary are largely based on habitat type, with *Z. capensis* beds and saltmarshes being identified as key habitats receiving a high sensitivity rating. These habitats are also particularly vulnerable to anthropogenic effects, with *Z. capensis* experiencing recent losses in Knysna Estuary (Wasserman et al. 2022). These losses have largely been linked to harmful macroalgal *Ulva lactuca* blooms (Pollard et al. 2018). *Ulva lactuca* macroalgae is associated with the successful uptake of inorganic nutrients and wide temperature tolerance (Wasserman et al. 2022). These macroalgal blooms are, therefore, common in areas where wastewater disposal occurs, introducing excessive inorganic nutrient into the water and in summertime conditions where the high temperature tolerance of the macroalgae favours growth of the macroalgae *U. lactuca* (Wasserman et al. 2022). This is especially noticeable in summer conditions within the Ashmead Channel region of the system, where wastewater discharge leads to high nutrient loads (Figure 5.1), which leads to algal blooms of *U. lactuca* (Human et al. 2016). The *U. lactuca* blooms lead to a thick green mat which covers and smothers the *Z. capensis* beds in the region (Wasserman et al. 2022). This, along with prolonged pressure from bait collecting, damage from boats and illegal bait digging (Claassens et al. 2020), have placed the crucial *Z. capensis* habitat under severe anthropogenic stress. The first recording of *U. lactuca* blooms was in the summer of 2014/2015 (Allanson et al. 2016, Human et al. 2016), after which *U. lactuca* blooms have also been noted in the Ashmead Channel during 2018 and 2021 (Wasserman et al. 2022).

Saltmarshes in Knysna Estuary have experienced an 18ha overall decrease between 2007 and 2019 (Claassens et al. 2020), largely due to anthropogenic developments (Wasserman et al. 2022). Developments such as bridges, roads, causeways, and seawalls lead to restricted tidal movement, which has severe effects on the saltmarsh habitats by impacting plant distribution patterns and limiting the deposition of detritus which these plants rely on for nutrients (Maree 2000). The National Water Act of 1998 (No. 36 of 1998) (Government Gazette No. 19182) and the National Environmental Integrated Coastal Management Act of 2008 (No. 24 of 2008) (Government Gazette No. 31884) require environmental impact assessment and authorisation in order to develop within the 5 m contour boundary of estuaries, which has reduced the impact on saltmarshes habitats in estuaries (Wasserman et al. 2022). Historic developments, however, continue to have detrimental effects on saltmarsh habitats as well as causing irreversible loss of historic saltmarsh habitats (Wasserman et al. 2022).

Figure 5.1: Camera footage illustrating high turbidity water due to high levels of eutrophication at site KN24 in the Ashmead Channel, Knysna Estuary

The identified high-sensitivity regions in the estuary have been zoned as quiet with the main channel zoned for high-intensity leisure (GRNP 2020). As such, a large proportion of the marine bay region of the system and the upper estuary regions are classified as high-sensitivity due to the widespread *Z. capensis* beds in the marine bay region, and intertidal salt flats in the upper estuary regions. The lagoon and lower estuary regions of the system are characterised by a wide variety of habitat types, with *Asparagopsis taxiformis* mixed and *Caulerpa filiformis* mixed habitats dominating the main river channel with widespread intertidal *Z. capensis* beds (GRNP 2020, Figure 5.2). The *Z. capensis* beds have received a high sensitivity rating, while the *A. taxifomis* and *C. filiformis* received a low sensitivity rating (Figure 5.2). Due to the widespread *C. filiformis* in this region, a large part of this region receives a low-sensitivity rating (Figure 5.2).

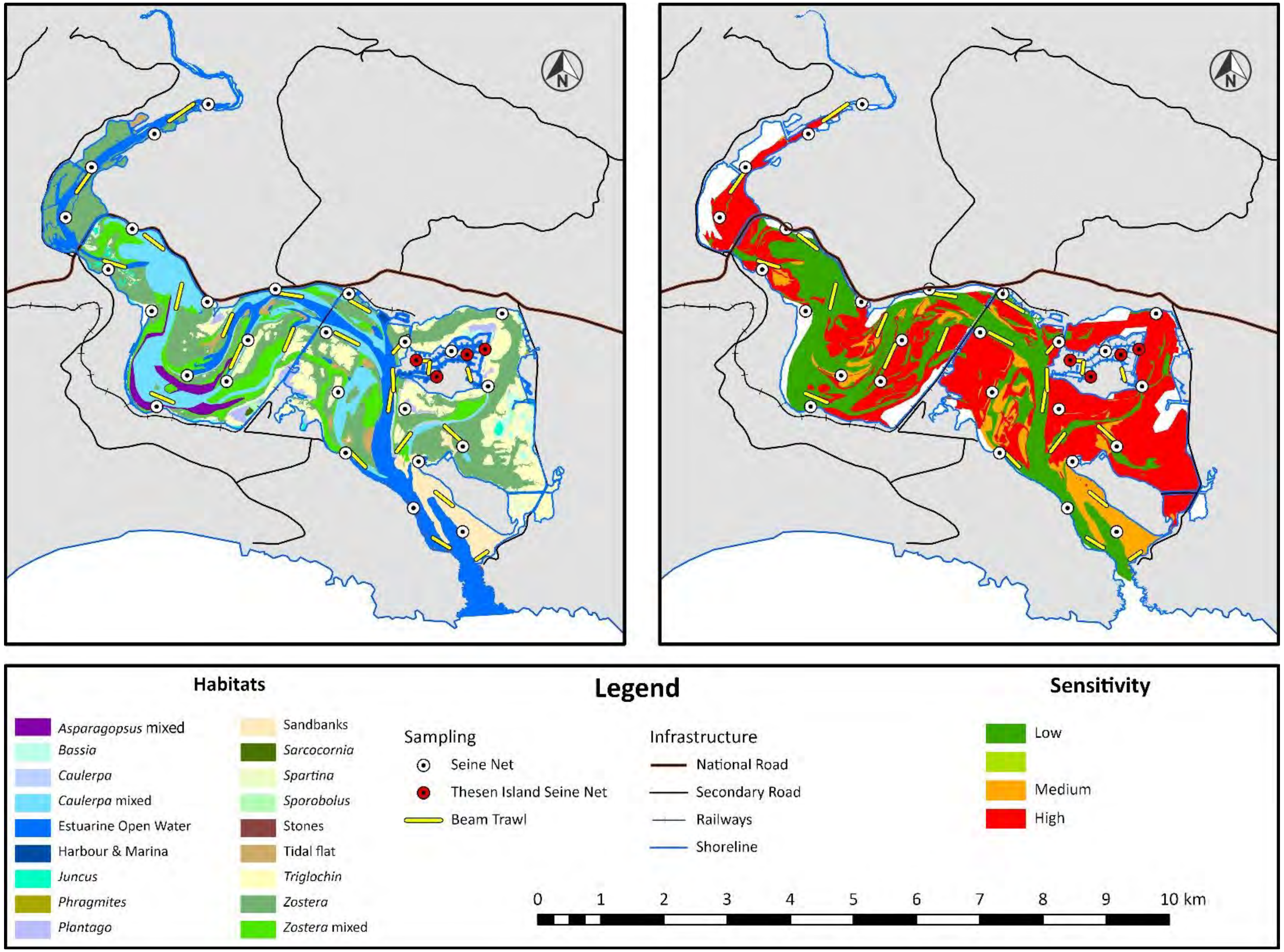


Figure 5.2: Map of current Knysna Estuary sensitivity zones linked to Knysna habitat (shapefiles provided by SANParks) with sampling sites superimposed onto the maps.

By utilising both seine net and beam trawl sampling methods, it was possible to sample a wide variety of habitats, with sample sites present in vegetated sites, sand banks, mud banks and open-water channels. The sites also covered a full variety of sensitivity zones (Figure 5.2) and special management zones (Figure 5.3). These special management zones are linked to sensitivity data, with key areas demarcated as special management regions within the Knysna Estuary (GRNP 2020, Figure 5.3), which put in place specific restrictions to a number of high sensitivity regions in the system.

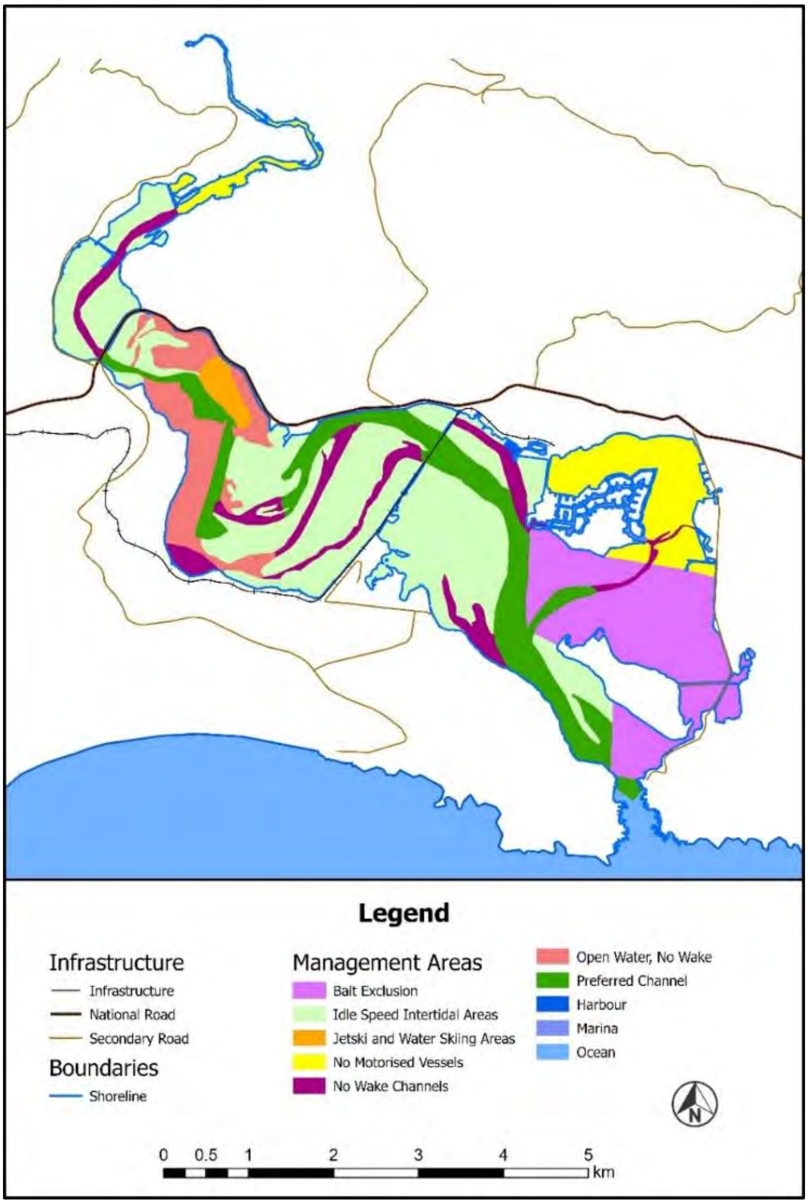


Figure 5.3: Current demarcated estuarine management areas in the Knysna Estuary (adapted from GRNP 2020).

5.2 Hotspot identification

5.2.1 Fishery species hotspot identification

The littoral fish assemblage (seine net sampling) dominated the fishery species captured during this study, with *Diplodus capensis, Lithognathus lithognathus, Pomatomus saltatrix, Rhabdosargus holubi, Sarpa salpa* and *Spondyliosoma emarginatum* sampled in large numbers. Fishery species were dominated by MEO species (*D. capensis, P saltatrix, S. salpa* and *S. emarginatum*) and MED species (*L. lithognathus* and *R. holubi*).

A large proportion of the fishery species sampled during this effort were juveniles; these fish are therefore below the size that they at which they recruit into the fishery and are able to be caught using rod and line fishing methods used by recreational and subsistence anglers (Table 5.1). Of the fishery species sampled *L. lithognathus* and *P. saltatrix* are of concern as both of these species are overexploited with *L. lithognathus* stocks collapsed (Table 5.1, Bennett and Lamberth 2012, Maggs and Mann 2012). A species which is known to have an important recreational fishery in the Knysna Estuary but did not appear is this sampling effort was *Pomadasys commersonnii,* this is another species of concern with an overexploitated stock status (Fennessy 2000). The species sampled in this effort that are not overexploited include *D. capensis, R. hobuli, S. salpa* and *S. emarginatum* (Table 2.1). Despite these species not being at risk of overexploitation, *D. capensis, R. holubi* and *S. salpa* form an important part of the subsistence fishery associated with the Knysna Estuary (Whitfield et al. 2022). These smaller-bodied fish species are widely caught by subsistence fishers and utilised as a food and protein source (Whitfield et al. 2022). *Spondyliosoma emarginatum* are usually associated with coastal subtidal reefs (Fairhurst 2007) is an unusual fishery species to be found in estuarine habitats. These fish also have a commercial value as they have become an important catch for commercial and recreational anglers due to the over-exploitation of many of the larger marine line fish species (Aitken and Mann 2000). Although the fishery within the Knysna Estuary is a conservation priority, the dominance of these MEO species highlights the importance of the Knysna Estuary for the coastal fisheries surrounding the estuary system, with only the MED species *L. lithognathus* and *R. holubi* being estuarine fishing species.

Table 5.1: Fishery species sampled using seine net (June 2021 and February 2022) and beam trawl (November 2021 and March 2022). CPUE (individuals/haul), fish length (mean and range TL mm), national size limits (TL mm) and the fishes current stock status

Fish species	**CPUE**	**Mean length (range) mm**	**National size limit (mm)**	**Current stock status**
Lithognathus lithognathus/ White Steenbras	0.9	179 (54-461)	600	<25%- collapsed
Pomatomus saltatrix/ Shad	0.9	123 (83-155)	300	25% - 40% - overexploited
Rhabdosargus holubi/ Cape Stumpnose	22.8	86 (15-355)	200	Not assessed
Diplodus capensis/ Blacktail	2.5	58 (12-313)	200	Not assessed
Sarpa salpa/ Strepie	2.5	134 (52-286)	150	>50% - underexploited
Spondyliosoma emarginatum/ Steenjie	5.5	65 (22-147)	0	Not assessed

Identifying key habitats of early juvenile fishery species is a key step in conserving these species and ensuring that fish are given the best opportunity to recruit into the adult population. The overall fishery species Kernel density was mapped in order to identify key habitats utilised by fishery species in the Knysna Estuary (Figure 5.4). The average catch from the five Thesen Island sites was calculated and plotted at the central Thesen Island site as described in Chapter 3 methods.

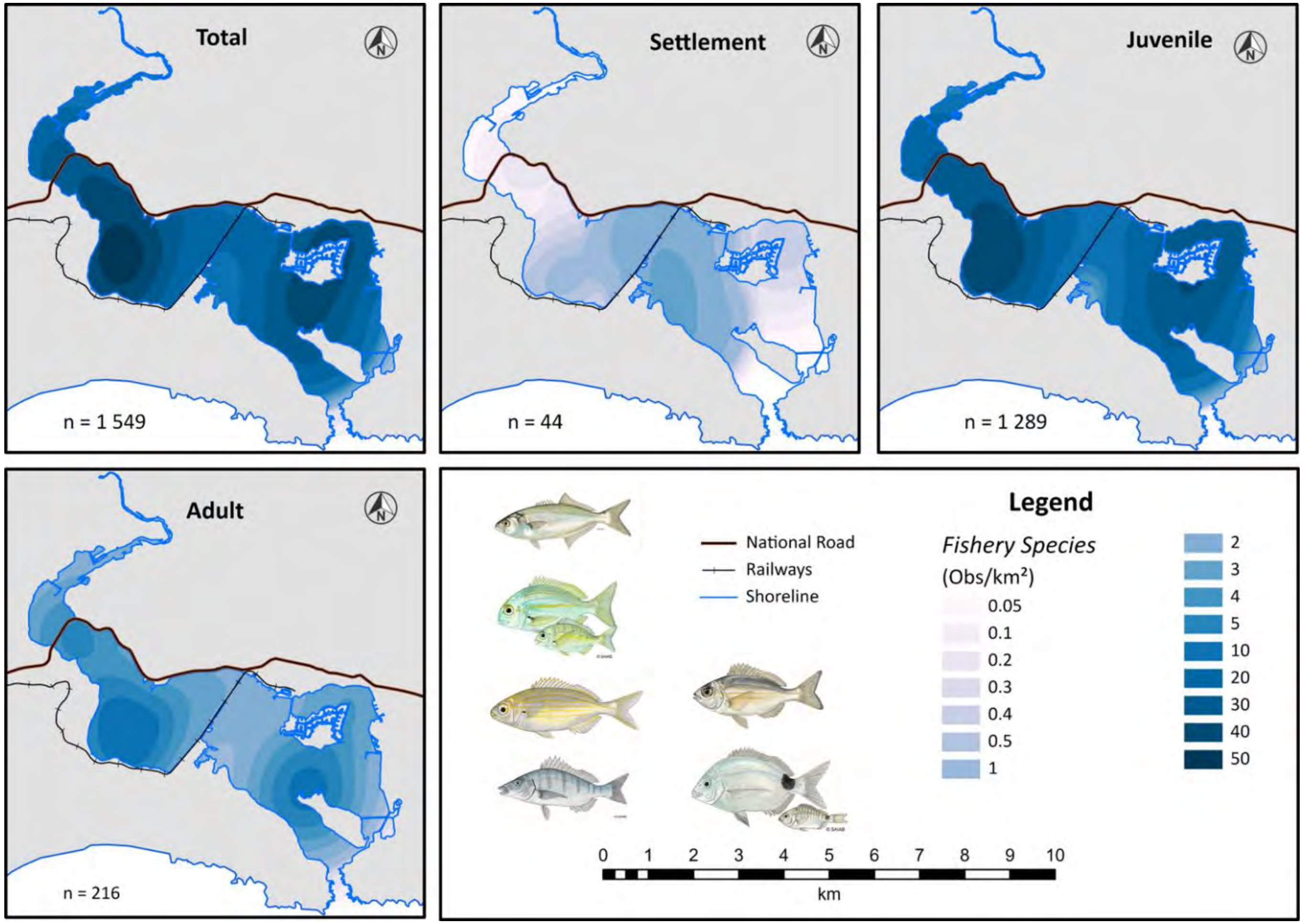


Figure 5.4: Kernel density distribution maps of the catch-per-unit-effort (CPUE) over two sampling trips (June 2021 and February 2022) for seine net sampled fishery species, *Pomatomus saltatrix, Spondylisoma emarginatum, Rhabdosargus holubi, Diplodus capensis, Sarpa salpa* and *Lithognathus lithognathus* (all species pictured). Densities represented as CPUE including total and settlement, juvenile and adult life stages sampled. n=catch numbers

Using spatial mapping of multiple fishery species in the Knysna Estuary, two areas were identified as critical nursery hotspots for seine net sampled key fishery species (Figure 5.4, Figure 5.5). These areas are the Ashmead Channel between Thesen Island and Leisure Island in the marine bay region (Figure 5.4, Figure 5.5), which was a hotspot for *L. lithognathus, S. salpa* and *S. emarginatum* (see Figures 3.8, 3.12, 3.13, Chapter 3). This area consists of a wide variety of habitats, encompassing areas of *Z. capensis, Z. capensis mixed, C. filiformis,* and the intertidal salt marsh plant *Triglochin bulbosa* (Figure 5.2). The abundance of *Z. capensis* and saltmarsh vegetation has meant that this area has, for the most part, been given a high sensitivity rating with the exception of the main channel, which is characterised by *C. filiformis* and therefore receives a low sensitivity rating (Figure 5.2). Along with the high sensitivity rating, much of the Ashmead channel falls within the bait exclusion special management area (Figure 5.3) which, along with protecting the bait resources of the area, reduces the negative impact the bait collecting practices have on the *Z. capensis* beds of this region. The Belvidere section on the west bank of the lagoon region was also identified as a hotspot (Figure 5.4, Figure 5.5), in particular for *L. lithognathus, P. saltatrix, R. holubi* and *S. salpa* (Figure 3.8, 3.10, 3.11, 3.12, Chapter 3). This area, however, currently has a low sensitivity rating due to *A. taxiformis* mixed and *C. filiformis* mixed habitat (Figure 5.2) being present. This important fishery species hotspot is therefore not currently recognised as an important conservation area. Low-sensitivity regions on the system are zoned for high-intensity leisure activities designated by the preferred channel (Figure 5.3, GRNP 2020), which can have highly detrimental effects on the habitats these fish species are reliant on in these key hotspots. The area around Belvidere in the lagoon region of the system is away from the main river channel and therefore receives very little boat traffic. The lack of boat traffic, access to a wide variety of littoral habitats with A. *taxiformis, C. filiformis* and *Z. capensis* in this area and higher water temperature (Table 2.3, Chapter 2) may explain the high catches of juvenile fisheries species in this area. Including this area as a high-sensitivity zone provides an opportunity to prioritise a zone which has been shown as an important nursery hotspot for fishery species that are currently overexploited and which contribute significantly to the small-scale, recreational and commercial fishery sectors.

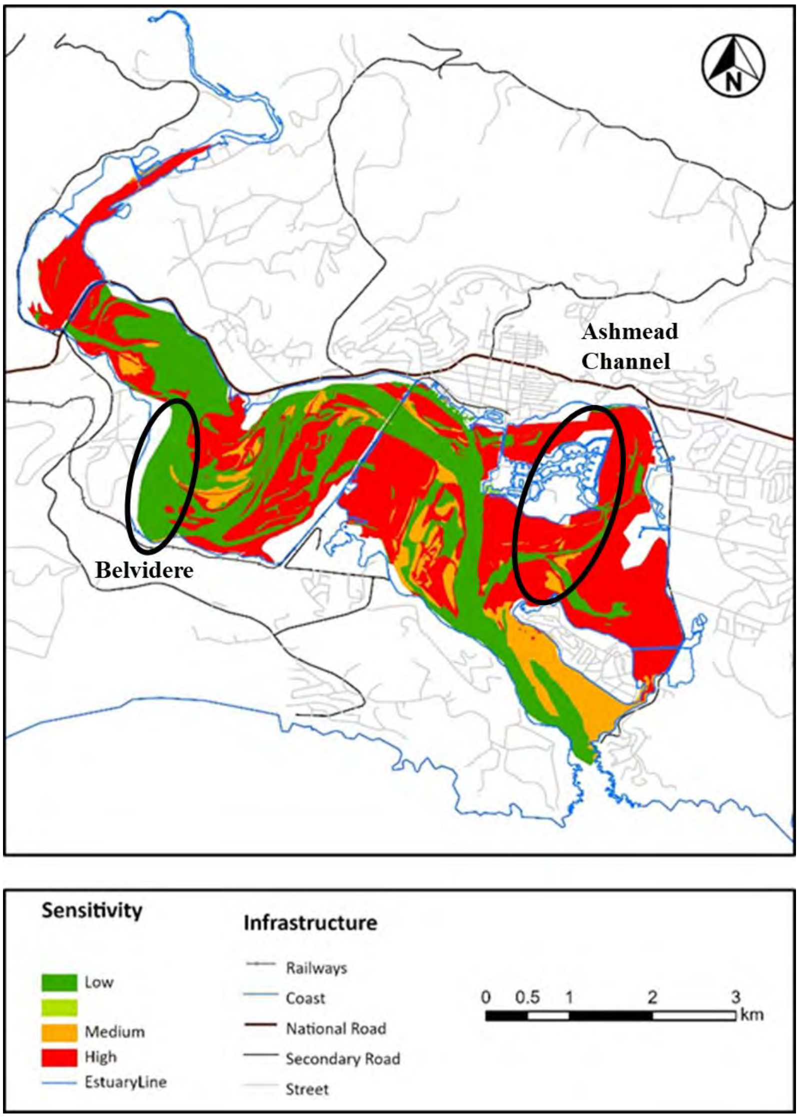


Figure 5.5: Current Knysna Estuary sensitivity map (GIS shapefiles provided by SANParks) with two critical fishery hotspots highlighted (Belvidere and Ashmead Channel)

5.2.2 Ecologically important species hotspot identification

Although fishery species usually garner much of the scientific attention due to their recreational and subsistence importance for people, these species tend to make up a small proportion of the overall fish assemblages in the Knysna Estuary. This means it is important to also consider the ecologically dominant fish species in estuaries in order to best conserve the overall nursery function of an estuary. In this regard, it is important to identify the smaller-scale patches that contribute the most to the overall population within the broad-scale nursery (Nagelkerken

2015). For this project, dominant littoral and demersal species from each functional estuarine guild were identified and highlighted as key ecological species. These species were *Atherina breviceps, Stolephorus holodon, Hyporhamphus capensis,* and Mugilidae spp. for seine net sampling (Chapter 3) and *Clinus superciliosus, Clinus spatulatus, Spondylisoma emarginatum,* and *Heteromycteris capensis* for beam trawl sampling (Chapter 4). The overall kernel densities for these ecologically important littoral (sampled using seine net) (Figure 5.6) and demersal (sampled using beam trawl) (Figure 5.7) fish species were mapped in order to identify key density hotspots within the Knysna Estuary system. For seine net species the average catch from the five Thesen Island sites was calculated and plotted at the central Thesen Island site as described in Chapter 3 methods.

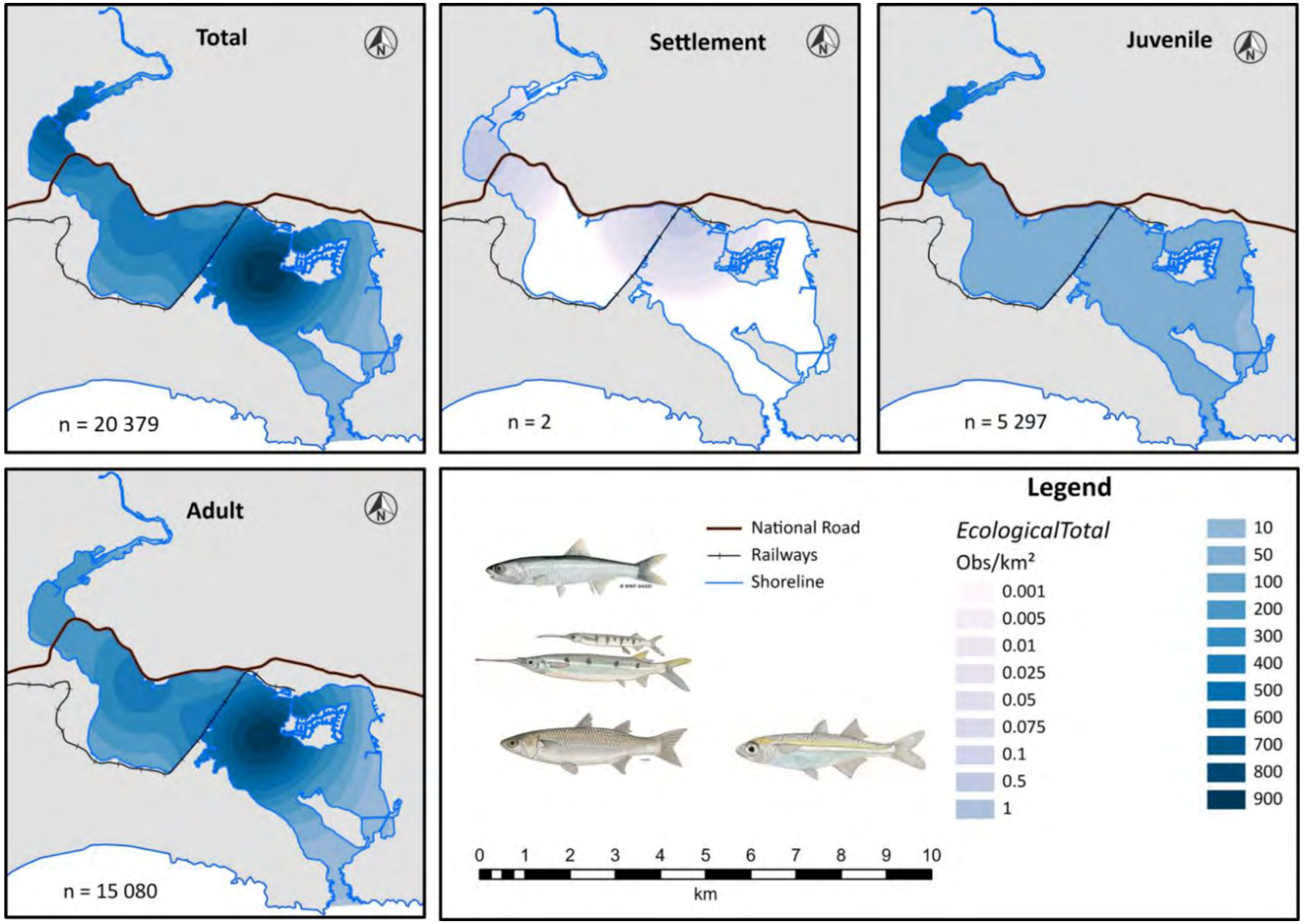


Figure 5.6: Kernel density spatial distribution maps of the catch-per-unit-effort (CPUE) over two sampling trips (June 2021 and February 2022) for ecologically important fish species sampled in the Knysna Estuary through seine netting. Species include *Atherina breviceps, Stolephorus holodon,* Mugilidae and *Hyporhamphus capensis* (all pictured). Including total, settlement, juvenile, and adult life stages. n = catch number

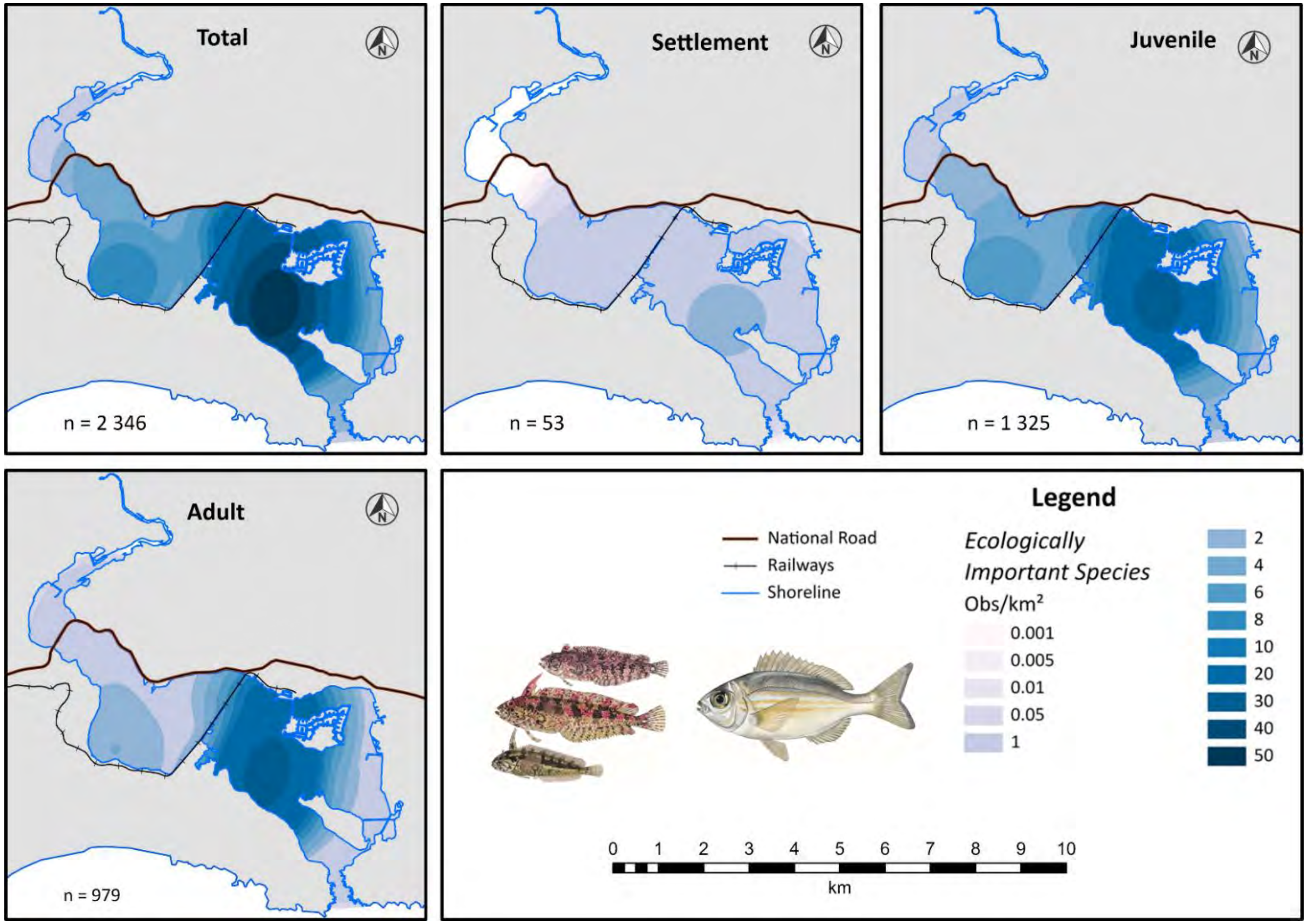


Figure 5.7: Kernel density spatial distribution maps of the average catch over two sampling trips (November 2021 and March 2022) for ecologically important fish species sampled in the Knysna Estuary by beam trawl. Species include *Clinus superciliosus* (pictured), *Clinus spatulatus* (not pictured), *Heteromycteris capensis* (not pictured), and *Spondylisoma emarginatum* (pictured). Including total, settlement, juvenile and adult life stages. n = catch number

Although there are areas within the current SANParks habitat-based sensitivity model (Figure 5.2) that were identified to be ineffective for predicting fishery species hotspots (e.g. Belvidere, Figure 5.4), when examining the spatial distribution of ecologically dominant species, it appears that the habitat-based sensitivity model corresponds with estuary-associated fish density hotspots. With dominant hotspots falling within high sensitivity regions for both the littoral (seine net sampled) fish species (Figure 5.6) and demersal (beam trawl sampled) species (Figure 5.7). However, the high densities of catches make it challenging to identify specific habitats within the hotspots. Seine net sampling in the observed density hotspot (Figure 5.6) at the head of Thesen Island occurred on shallow *Z. capensis* beds, which all receive a high sensitivity rating. The beam trawl sampling in the density hotspot at the head of Ashmead Channel, between Leisure Island and Thesen Island (Figure 5.7), would, for a large part, have been undergone in open water on the fringes of the *Z. capensis* beds, due to the inefficiency and destructive nature of this sampling method for vegetated sites. As a result, the areas sampled using beam trawl will therefore fall into the low sensitivity estuarine open water, and *C. filiformis* habitats found adjacent to the high sensitivity *Z. capensis* habitats in this region of the system (Figure 5.2). This highlights the importance of sampling both the littoral and demersal fish assemblage and the overall habitat mosaic found within estuarine habitats, as very few species are confined to a single nursery habitat (Nagelkerken 2007), but instead use a mosaic of habitats on a daily basis (Boström et al. 2011).

Settlement stage fish have been shown to show low levels of habitat association due to the risk associated with searching in the water column for a preferred habitat (Grol et al. 2011, Nagelkerken et al. 2015). As a result, settlement stage larvae will often occupy the first available suitable habitat when entering estuaries from the open ocean (Grol et al. 2011, Nagelkerken et al. 2015). This means that settlement stage fish will often associate with less structurally complex habitats such as sandy bottoms, macroalgal clumps or dead coral (Dahlgren and Eggleston 2000). In terms of life stage, the current SANParks habitat-based sensitivity model is largely inefficient for protecting these highly vulnerable settlement stage fishes. This is evident by the low catches of ecologically important seine net and beam trawl settlement stage samples when compared to the other life stages (Figure 5.6, Figure 5.7). While a noticeable hotspot was observed in the beam trawl settlement stage catches at the head of Leisure Island (Figure 5.7), this hotspot appears to be the centre of distribution rather than a hotspot associated with a key habitat type, with a broad distribution surrounding the hotspot (Figure 5.7).

5.3 Conservation implications, recommendations and conclusion

Developing fish specific conservation methods in Knysna Estuary is difficult as the system, along with having a high conservation importance, is also of recreational and subsistence fishery importance (Whitfield et al. 2022). As important as conserving the ecology within the system is, it is important to ensure that conservation efforts do not compromise the associated recreational fishery and its economic benefit. Ensuring that fishing in the Knysna Estuary is sustainable is crucial to ensuring the long-term ecological and economic benefits of the estuarine system (Whitfield et al. 2022). There is currently not any spatial protection for fish species in the Knysna Estuary, with recreational fish managed according to national size and bag limits. Unfortunately, these measures do very little to conserve fish due to the wide-scale non-compliance with legislation (Hauck and Kroese 2006). Estuarine habitats can act as "sources" or "sinks" for adult populations of fishery species (Secor and Rooker 2005). In this regard, some estuarine fish populations are considered "sources" of recruits to adult and fishery populations and highlights the importance of connectivity links between estuaries and the adjacent marine environments (Gillanders et al. 2012). To ensure that the Knysna Estuary acts as a "source" for adult populations for fishery species, it is critical that the identified hotspot at Belvidere and in the Ashmead Channel for juveniles of estuarine and coastal fishery species acquires adequate protection.

There is a complex system of biotic and abiotic factors that come into play when understanding the spatial distribution of fishes and how fish species make use of estuaries during their life cycle (Whitfield 1998, Strydom 2015, Nashima et al. 2021). When considering the wider biotic components in the system, protecting crucial habitats in the Knysna Estuary should still be a priority. *Zostera capensis* is widely used by many fish species in the system, being the most widespread habitat type in the Knysna Estuary and frequently utilised habitat by ecologically dominant fish species. The marine bay region of the system is dominated by *Z. capensis* (Figure 5.2) and plays host to the overall ecologically important species density hotspots for both seine net and beam trawl fish (Figure 5.7 and Figure 5.8). In recent years the *Z. capensis* beds in

Knysna have been placed under severe pressure due to anthropogenic stress as a result of eutrophication leading to harmful algal blooms (Pollard et al. 2018). This is especially noticeable in the Ashmead Channel region of the system, where protracted problems with the Knysna Municipal wastewater treatment plant have contributed towards high nutrient loads, which leads to algal blooms of *Ulva lactuca* (Figure 5.5) (Human et al. 2016). This, along with prolonged pressure from bait collecting, damage from boats and illegal bait digging, have placed this crucial habitat under severe anthropogenic stress (Claassens et al. 2020). This area of the Knysna Estuary was identified as a critical habitat for important fishery species (Figure 5.4).

The lack of implementation of the current national fishery legislation has resulted in continued low compliance by fishers (Brouwer et al. 1997). This project provides the information to highlight key areas utilised by early juveniles of fishery species in the Knysna Estuary. Although these smaller juvenile fish are at a lower risk of exploitation as they are not targeted by traditional recreational and subsistence angling methods, ensuring that these fish are protected and given the best opportunity to recruit into the adult population ensures the continued sustainability of the fishery in the estuary and surrounding marine waters. Although Knysna Estuary does have formal protection, implementing management measures within the Knysna Estuary is made difficult due to the fact that the Knysna Estuary is an open access system (Claassens et al. 2020). This open access makes the system vulnerable to a wide variety of anthropogenic impacts including resource exploitation, pollution, and habitat alteration.

Protecting the key juvenile hotspots (i.e. Belvidere hotspot and Ashmead channel hotspot, Figure 5.4) identified for fishery species is critical to ensuring the continued sustainability of the Knysna Estuary. The Ashmead Channel hotspot is currently relatively well protected due to the high sensitivity rating (Figure 5.2) and the bait exclusion region (Figure 5.3), which reduces the anthropogenic stress on the *Z. capensis* habitats in this area. The excessive nutrients entering the Ashmead Channel and the associated *U. lactuca* blooms in this region place huge stress on the critical *Z. capensis* habitats and minimising the effects these green tides have on this region of the system needs to be a priority to ensure this critical hotspot remains ecologically functional. The identified Belvidere fishery species hotspot is, however not currently identified as a high sensitivity area receiving a low sensitivity rating. This area currently does not receive a large amount of anthropogenic pressure as it is on the opposite bank from the main town of Knysna, limiting the access for many recreational and subsistence anglers and reducing the amount of bait collecting that occurs in this area. This region currently falls under the “No Wake Channel” and “Open Water, No Wake” special management area (Figure 5.3) and is situated away from the preferred channel on the system, which reduces the damage to habitats associated with high-intensity leisure activities. This area is, therefore, not placed under major anthropogenic stress, which is likely a contributing factor to the fishery species' usage of this area. Ensuring that future anthropogenic stress is minimised should be a priority for conserving the fishery species making use of this habitat.

The Knysna Estuary is in a relatively unique position of being managed by SANParks, which allows for the opportunity to set up the best management strategies possible to ensure conservation is a priority in the system. SANParks has also shown a willingness to accept recommendations based on scientific evidence with the overall goal of ensuring the longevity of services offered by this critical estuary system (Scheepers et al. 2011). Management measures are however difficult to implement in the Knysna Estuary due to the fact the system is open access which makes the system particularly vulnerable to resource exploitation (Claassens et al. 2019). This study highlights the importance of monitoring fish assemblages in estuarine habitats and, in particular, the use of spatial mapping, which allows key areas to be identified and highlighted for conservation purposes within the complex mosaic of habitats used by fish species in estuaries.

The major recommendations for fish-specific conservation based on the current study would be to prioritise the area around Belvidere in the lagoon. This area was highlighted as a hotspot for juvenile fishery species and currently is not viewed as a high-sensitivity zone (Figure 5.5). The Ashmead Channel is a region which has also been highlighted as a key fishery species juvenile hotspot but is currently relatively well protected with a high sensitivity rating and a bait exclusion region. Reducing the high nutrient input due to wastewater in this area needs to become a priority in order to ensure the stability of the *Z. capensis* habitat and, therefore, the continued functioning of this region as an important nursery hotspot.

Estuaries are complex and ever-changing environments, and therefore continued research is important to update and gain a better long-term understanding of how fish species are using the Knysna Estuary. A recommendation would therefore be to set up a long-term monitoring programme on this estuary to gain a better understanding of the spatial distribution of fishes in the system. More frequent spatial and temporal sampling may also assist in capturing important species which are known to be present in the system, such as the fishery species *Pomadasys commersonnii,* but which was not present in these sampling efforts. The only previous widespread long-term sampling effort in the Knysna Estuary was that of DFFE (2007 – 2012) which made use of biannual sampling efforts, and it would be advised that any further long-term sampling efforts mirror this. Biannual sampling efforts with a winter and summer sample set give the best opportunity to properly understand what fish species are entering and make use of the Knysna Estuary system which is the allows for measures to be put into place to protect these fish.

Estuaries play a critical role in the life cycles of many fish species with many species reliant on these systems for their survival. These estuaries are however extremely vulnerable to anthropogenic stress with increasing populations in coastal regions placing these systems under severe pressure. In large estuary systems such as the Knysna Estuary, protecting the whole estuary is logistically not a possibility, identifying key habitats using spatial data within the estuary therefore allows for a targeted approach to protecting the fish species utilising these estuarine habitats, while still allowing people to make use of estuaries for recreation and subsistence. The data provided in this study showcases the potential for spatial analysis and key hotspot identification within an estuary in order to protect important habitats for fish species utilising estuarine systems.

REFERENCES

Adams JB. 2016. Distribution and status of *Zostera capensis* in South African estuaries — A review. *South African Journal of Botany* 107:63-73.

Adams J, Fernandes M, Riddin T. 2019. *Chapter 5: Estuarine Habitat extent and trend in South African National Biodiversity Assessment 2018: Technical Report. Volume 3: Estuarine Realm*. South African National Biodiversity Institute, Pretoria.

Aitken AP, Mann BQ. 2000. Steentjie (*Spondyliosoma emarginatum*). In: Mann BQ (ed). Southern African Marine Linefish Status Reports. Special Publication of the Oceanographic Research Institute, South Africa 7: 191–192.

Allanson BR, Human LRD, Claassens L. 2016. Observations on the distribution and abundance of a green tide along an intertidal shore, Knysna Estuary. *South African Journal of Botany* 107:49-54.

Bailey SE, James NC. 2013. Fish sampling in the marine dominated Kariega Estuary, South Africa, using a demersal otter trawl: day/night effects. *African Journal of Aquatic Science* 38:1, 115-12.

Barletta M, Costa MF. 2009. Living and Non-living Resources Exploitation in a Tropical Semi-arid Estuary. *Journal of Coastal Research* 56:371-375.

Barnes RSK. 2010. Spatial variation in abundance and diversity of the smaller surface and near-surface eelgrass-associated intertidal macrobenthos within a warm-temperate estuarine bay in the Garden Route National Park, RSA. *Aquatic Conservation: Marine and Freshwater Ecosystems* 20:762-772.

Bennett RH, Lamberth SJ. 2012. White Steenbras (*Lithognathus lithognathus*). In: Mann BQ (Ed). Southern African Species Profiles, Special Publication, Oceanographic Research Institute, Durban 9:239-240

Bishop JM, Moore ABM, Alsaffar AH, Abdul Ghaffar AR. 2015. The distribution, diversity and abundance of elasmobranch fishes in a modified subtropical estuarine system in Kuwait. *Journal of Applied Ichthyology* 32(1): 75-82.

Blaber SJM. 1974. Field studies of the diet of *Rhabdosargus holubi* (Pisces: Teleostei: Sparidae). *Journal of Zoology* 173(3): 407-417.

Blaber SJM, Blaber TG. 1980. Factors affecting the distribution of juvenile estuarine and inshore fish. *Journal of Fish Biology* 17(2): 143-162.

Boström C, Pittman SJ, Simenstad C, Kneib RT. 2011. Seascape ecology of coastal biogenic habitats: advances, gaps, and challenges. *Marine Ecology Progress Series* 427:191–217.

Bulpin TV. 1978. *Readers Digest illustrated guide to Southern Africa*. Cape Town, Readers Digest Vol 102-107.

Bulleri F, Airoldi L. 2005. Artificial marine structures facilitate the spread of a nonindigenous green alga, *Codium fragile* ssp. *tomentosoides*, in the North Adriatic Sea. *Journal of Applied Ecology* 42:1063–1072.

Burchard H, Baumert H. 1998. The formation of estuarine turbidity maxima due to density effects in the salt wedge. A hydrodynamic process study. *Journal of Physical Oceanography* 28:309-321.

Butler EC, Childs A-R, Smith MKS, Foster RM, Potts WM. 2022. Spawning observations of *Pomadasys commersonnii* in the marine section of the Knysna estuarine bay, Western Cape, South Africa. *African Journal of Marine Science* 44: 101-106.

Bruton MN. 1995. Have fishes had their chips? The dilemma of threatened fishes. *Environmental Biology of Fishes* 43:1 – 27.

Brouwer SL, Mann BQ, Lamberth SJ, Sauer WHH, Erasmus C. 1997. A survey of South African the shore-angling fishery. *South African Journal of Marine Science* 18:165-177.

Carl WC. 2009. Coastal fish- fish of the open sea floor. the Encyclopedia of New Zealand. Updated 2 March 2009.

Claassens L. 2016. An artificial water body provides habitat for an endangered estuarine seahorse species. *Estuarine, Coastal and Shelf Science* 180:1–10.

Claassens L, Harasti D. 2020. Life history and population dynamics of an endangered seahorse (*Hippocampus capensis*) within an artificial habitat. *Journal of Fish Biology* 97(4): 974-986.

Claassens L, Barnes RSK, Wasserman J, Lamberth SJ, Miranda NAF, van Niekerk L, Adams JB. 2020. Knysna Estuary health: ecological status, threats and options for the future. *African Journal of Aquatic Science* 45:1-2, 65-82.

Colloca F, Garofalo G, Bitetto I, Facchini MT, Grati F, Martiradonna A, Mastrantonio G, Nikolioudakis N, Ordinas F, Scarcella G, Tserpes G, Tugore MP, Valavanis V, Carlucci R, Fiorention F, Follesa MC, Iglesias M, Knittweis L, Lefkaditou E, Lembo G, Manfredi C, Massuti E, Pace ML, Papadopoulou N, Sartor P, Smith CJ, Spedicato TM. 2015. The Seascape of Demersal Fish Nursery Areas in the North Mediterranean Sea, a First Step Towards the Implementation of Spatial Planning for Trawl Fisheries. *PLoS ONE* 10(3): e0119590.

Costa MDP, Possingham HP, Muelbert, JH. 2016. Incorporating early life stages of fishes into estuarine spatial conservation planning. *Aquatic Conservation: Marine and Freshwater Ecosystems* 26: 1013– 1030.

Costa MDP, Magris RA, Muelbert JH, Vieira JP, Castello JP, Copertino, Possingham HP. 2022. Spatial zoning to conserve fish species with complex life cycles in estuaries. *Ocean and Coastal Management* 221.

Daigle RM, Metaxas A, Balbar AC, McGowan J, Treml EA, Kuempel CD, Possingham HP, Maria Begeet. 2020. Operationalizing ecological connectivity in spatial conservation planning with Marxan Connect. *Methods Ecology and Evolution* 11: 570– 579.

Day JH. 1981. *Summaries of current knowledge of 43 estuaries in southern Africa*. In: Estuarine Ecology with Particular References to Southern Africa 251-330. Balkema, Capetown.

Day JH, Millard NAH, Harrison AD. 1952. The ecology of South African estuaries. Part 3. Knysna: a clear open estuary. *Transactions of the Royal Society of South Africa* 33: 367–413.

Deacon N, Hecht T. 1995. Observations on the thermoregulatory behaviour of juvenile spotted grunter, *Pomadasys commersonnii* (Haemulidae: Pisces). *Journal of Applied Ichthyology* 11:100-110.

Deacon N, Hecht T. 1996. The effect of temperature and photoperiod on the growth of juvenile spotted grunter, *Pomadasys commersonnii* (Haemulidae: Pisces). *South African Journal of Marine Science* 17:55-60.

de Villiers NM, Barker C, Claassens L, Hodgson AN. 2019. Conservation value of *Codium tenue* habitat for the endangered Knysna seahorse *Hippocampus capensis*. *Journal of Fish Biology* 95: 1457– 1464.

Enviro-Fish Africa. 2007. C.A.P.E. estuaries management programme. *Gamtoos Estuary management plan situation assessment* 1.

Fairhurst L, Attwood CG, Durholtz MD, Moloney CL. 2007. Life history of the steentjie *Spondyliosoma emarginatum* (Cuvier 1830) in Langebaan Lagoon, South Africa. *African Journal of Marine Science* 29(1): 79-9.

Fennessy ST. 2000. Stock assessment and management of spotted grunter in KwaZulu-Natal. Final Report submitted to Department of Environmental Affairs and Tourism, Branch: Marine and Coastal Management, 8p.

Garden Route National Park (GRNP). 2020. *Draft Park Management Plan 2020-2029.*

Gillanders BM, Elsdon TE, Roughan M. 2012. Connectivity of estuaries. In: Heip CHR, Philippart CJM, Middelburg JJ (Eds). Functioning of Estuaries and Coastal Ecosystems. Elsevier: Amsterdam.

Grange N. 1992. *The Influence of Contrasting Freshwater Inflows on the Feeding Ecology and Food Resources of Zooplankton in Two Eastern Cape Estuaries, South Africa*. Ph.D. Thesis Rhodes University. Grahamstown. Unpublished report.

Grindley JR. 1985. *Estuaries of the Cape, Part II: Synopses of available information on individual systems. Knysna (CMS13).* Report No. 30. CSIR Research Report 429: 80.

Guest MA, Connoll RM, Loneragan NR. 2003. Seine nets and beam trawls compared by day and night for sampling fish and crustaceans in shallow seagrass habitat. *Fisheries Research* 64(2–3): 185-196.

Hauck M, Kroese M. 2006. Fisheries compliance in South Africa: a decade of challenges and reform 1994-2004. *Marine Policy* 30:74-83.

Hanekom N, Hutchings L, Joubert PA, van der Byl PCN. 1989. Sea temperature variations in the Tstsikamma Coastal National Park, South Africa, with notes on the effect of cold conditions on some fish populations. *South African Journal of Marine Science* 8: 145-153.

Harrison TD. 2002. Preliminary assessment of the biogeography of fishes in South African estuaries. *Marine and Freshwater Research* 53: 479–490

Harrison TD. 2005. Ichthyofauna of South African estuaries in relation to the zoogeography of the region. *Smithiana Bulletin* 6: 1-27.

Harrison, TD, Whitfield AK. 1995. Fish community structure in three temporarily open/closed estuaries on the natal coast. *Ichthyological bulletin of the J.L.B Smith institute of ichthyology* 64:80.

Harrison TD, Armour ND, McNeill MT, Moorhead PW. 2017. A comparative study of Northern Ireland's estuaries based on the results of beam trawl fish surveys. *Estuarine, Coastal and Shelf Science* 198: 172-182.

Hemingway KL, Elliott M. 2002. Field methods. *Fishes in Estuaries* 410-509.

Holleman W, von der Heyden S, Zsilavecz G. 2012. Delineating the fishes of the *Clinus superciliosus* species complex in southern African waters (Blennioidei: Clinidae: Clinini), with the validation of *Clinus arborescens* Gilchrist & Thompson, 1908 and *Clinus ornatus* Gilchrist & Thompson, 1908, and with descriptions of two new species. *Zoological Journal of the Linnean Society* 166: 827-853.

Human LRD, Adams JB, Allanson BR. 2016. Insights into the cause of an *Ulva lactuca* Linnaeus bloom in the Knysna Estuary. *South African Journal of Botany* 107 55-62.

James NC, Harrison TD. 2008. A preliminary survey of the estuaries on the south coast of South Africa, Cape St Blaize, Mossel Bay - Robberg Peninsula, Plettenberg Bay, with particular reference to the fish fauna. *Transactions of the Royal Society of South Africa* 63(2): 111-127.

James NC, Leslie TD, Potts WM, Whitfield AK, Rajkaran A. 2019. The importance of different juvenile habitats as nursery areas for a ubiquitous estuarine-dependent marine fish species. *Estuarine, Coastal and Shelf Science* 226.

Johnson DD, Rotherham D, Gray CA. 2008. Sampling estuarine fish and invertebrates using demersal otter trawls: Effects of net height, tow duration and diel period. *Fisheries Research* 93: 315-323.

Keur R, James N, Rajkaran A. 2019. A tale of two habitats: preliminary comparison of fish abundance and diversity between saltmarsh- and mangrove-dominated creeks in the Nahoon Estuary, South Africa. *African Journal of Marine Science* 41(4):449-454.

Knysna Municipality. 2009. *Medium Term Revenue and Expenditure Framework 2009/10 to 2011/12. Review of the Knysna Integrated Development Plan.* Municipality of Knysna: Knysna.

Koen JH, Crowe TM. 1987. *Animal-habitat relationships in the Knysna Forest, South Africa: discrimination between forest types by birds and invertebrates.* South African Forestry Research Institute, Saasveld Forestry Research Centre. South Africa.

Kuipers BR, Maccurin B, Miller JM, Van Der Veer HW, Witte JIJ. 1992. Small Trawls in Juvenile Flatfish Research: Their Development and Efficiency. *Netherlands Journal of Sea Research* 29:109-117.

Kikuchi E, De Grande FR, Duarte RM, Vaske-Júnior T. 2019. Thermal response of demersal and pelagic juvenile fishes from the surf zone during a heat-wave simulation. *Journal of Applied Ichthyology* 35: 1209– 1217.

Lagabrielle E, Lombard AT, Harris JM, Livingstone TC. 2018. Multi-scale multi-level marine spatial planning: A novel methodological approach applied in South Africa. *PLoS ONE* 13(7): e0192582.

Lamberth JL, Turpie JK. 2003. The role of estuaries in South African fisheries: Economic importance and management implications. *African Journal of Marine Science* 25: 131-157.

Largier JH, Attwood C, Harcourt-Baldwin J-L. 2000. The hydrographic character of the Knysna Estuary. *Transactions of the Royal Society of South Africa* 55(2):107-122.

Leslie T, James NC, Potts WM, Rajkaran A. 2017. The relationship between habitat complexity and nursery provision for an estuarine-dependent fish species in a permanently open South African estuary. *Estuarine, Coastal and Shelf Science* 198:183−192.

Le Quesne W. 2000. Nekton utilisation of intertidal estuarine marshes in the Knysna Estuary. *Transactions of the Royal Society of South Africa* 55(2):205-214.

Mackelworth, P. 2012. Peace parks and transboundary initiatives: implications for marine conservation and spatial planning. *Conservation Letters* 5: 90-98.

Maggs JQ, Mann BQ. 2012. Elf (*Pomatomus saltatrix*). In: Mann BQ (Ed). Southern African Species Profiles, Special Publication, Oceanographic Research Institute, Durban 9:239-240

Maree B. 2000. Structure and status of the intertidal wetlands of the Knysna Estuary. *Transactions of the Royal Society of South Africa* 55: 163-176.

Marker ME. 2003. The Knysna Basin, South Africa: geomorphology, landscape sensitivity and sustainability. *The Geographical Journal* 169(1): 32-42.

Marshall S, Elliot M. 1998. Environmental influence on the fish assemblage of the Humber Estuary, UK. *Estuarine, Coastal and Shelf Science* 46:175-184.

Nagelkerken I. 2007. Are non-estuarine mangroves connected to coral reefs through fish migration? *Bulletin of Marine Science* 80:595–607.

Nagelkerken I, Sheaves M, Baker R, Connolly RM. 2015. The seascape nursery: a novel spatial approach to identify and manage nurseries for coastal marine fauna. *Fish and Fisheries* 16: 362-371.

Nashima, FP, Strydom NA, Lamberth SJ. 2021. Abundance and Diversity of Fish Assemblages Along the River-Estuary Continuum in a Fluvially Dominated Southern African Coastal System. *Estuaries and Coasts* 44: 2291–2306.

Nodo P. 2016. *Distribution and abundance of demersal fish species, with emphasis on dusky kob and spotted grunter, in the Kariega and Great Fish Estuaries*. Master of Science. Walter Sisulu University. South Africa.

Nodo P. 2021. *Demersal fish distribution in the shallow marine nearshore and estuarine seascape of Algoa Bay: Nursery areas and the effect of environmental drivers*. Doctor of Philosophy. Rhodes University. South Africa.

Nodo P, James NC, Childs AR, Nakin MDV. 2017. The impact of river flooding and high flow on the demersal fish assemblages of the freshwater dominated Great Fish Estuary, South Africa. *African Journal of Marine Science* 39(4): 491-502.

Nodo P, James NC, Childs AR, Nakin MDV. 2018. Response of demersal fish assemblages to an extreme flood event in a freshwater-deprived estuary in South Africa. *Marine and Freshwater Research* 69: 253-266.

North EW, Houde ED. 2003. Linking ETM physics, zooplankton prey, and fish early-life histories to striped bass *Morone saxatilis* and white perch *M. americana* recruitment. *Marine Ecology Progress Series* 260: 219-236.

Paterson AW, Whitfield AK. 1996. Fish associated with a salt marsh creek in the Kariega Estuary, South Africa. *Transactions of the Royal Society of South Africa* 21:195-218.

Petersen R, Govender D, Smith K, Russell I. 2020. Standard operating procedures for the capture, scientific manipulation, aneasthesia and euthanasia of fish in South African National Parks.

Petrik R, Levin PS. 1999. Estimating relative abundance of seagrass fishes: a quantitative comparison of three methods. *Environmental Biology of Fishes* 58: 461-466.

Piercer C, Rasmussen J, Leggett W. 1990. Sampling littoral fish with a seine: corrections for variable capture efficiency. *Canadian Journal of Fisheries and Aquatic Sciences* 47: 1004-1810.

Pollard M, Hodgson AN, Kok HM, Whitfield AK. 2017. Eelgrass beds and bare substrata – sparid and mugilid composition in contrasting littoral estuarine habitats. *African Journal of Marine Science* 39(2): 211-224.

Pollard M, Whitfield AK, Hodgson AN. 2018. Possible influences of a macroalgal bloom in eelgrass beds on fish assemblages in the lower Knysna Estuary, South Africa. *African Journal of Aquatic Science* 43(3):319-323.

Pomfret JR, Turner GS, Phillips S. 1988. Beam trawl surveys as a monitoring tool in polluted estuaries in north-east England. *Journal of Fish Biology* 33:71-77.

Potts WM, Götz A, James N. 2015. Review of the projected impacts of climate change on coastal fishes in southern Africa. *Reviews in Fish Biology and Fisheries* 25:603–630.

Potts WM, Saayman M, Saayman A, Mann BQ, Van der Merwe P, Britz P, Bova C. 2022. Understanding the economic activity generated by recreational fishing in South Africa provides insights on the role of recreational fisheries for social development. *Fisheries Management and Ecology* 29:29– 43.

Pressey RL, Cabeza M, Watts ME, Cowling RM, Wilson KA. 2007. Conservation planning in a changing world. *Trends in Ecology & Evolution* 22(11):583-592.

Prestrelo L, Vianna M. 2016. Identifying multiple-use conflicts prior to marine spatial planning: A case study of A multi-legislative estuary in Brazil. *Marine policy* 67: 83-93.

Richardson N, Whitfield AK, Paterson AW. 2006. The influence of selected environmental parameters on the distribution of the distribution of dominant demersal fishes in the Kariega Estuary channel, South Africa. *African Zoology* 41:89-102.

Scheepers K, Swemmer LK, Vermeulen WJ. 2011. Applying adaptive management in resource use in SANParks. *Koedoe* 53:144-157.

Schmidt J. 2013. *Influence of Predicted Sea-level Rise on the Salt Marsh of the Swartkops, Kromme and Knysna Estuaries*. MSc Thesis. Nelson Mandela Metropolitan University. Unpublished Report.

Schumann E. 2000. Oceanic exchanges and temperature variability in the Knysna Estuary. *Transactions of the Royal Society of South Africa* 55(2):123-128

Secor H, Rooker J. 2005. Connectivity in the life histories of fishes that use estuaries. *Estuarine Coastal and Shelf Science* 64:1-3

Selleslagh, J, Amara R. 2008. Environmental factors structuring fish composition and assemblages in a small macrotidal estuary (eastern English Channel). *Estuarine, Coastal and Shelf Science* 79(3):507-517.

Selwood KE, Wintle BA, Kujala H. 2019. *Collaborative conservation planning: Quantifying the contribution of expert engagement to identify spatial conservation priorities*. Conservation Letters.

South African National Parks. 2017. *Garden Route National Park: State of Knowledge*. South African National Parks, Unpublished report.

Shokri MR, Gladstone W. 2013. Limitations of habitats as biodiversity surrogates for conservation planning in estuaries. *Environmental Monitoring Assessing* 185: 3477–3492.

Strydom NA. 2015. Patterns in larval fish diversity, abundance, and distribution in temperate South African estuaries. *Estuaries and Coasts* 38 (1): 268–284.

Thiel R, Cabral H, Costa MJ. 2003. Composition, temporal changes and ecological guild classification of the ichthyofaunas of large European estuaries – a comparison between the Tagus (Portugal) and the Elbe (Germany). *Journal of Applied Ichthyology* 19: 330-342.

Turpie JK, Adams JB, Joubert A, Harrison TD, Colloty BM, Maree RC, Whitfield AK, Wooldridge TH, Lamberth SJ, Taljaard S, Van Niekerk L. 2002. Assessment of the conservation priority status of South African estuaries for use in management and water allocation. *Water SA* 28(2): 191-206

Vanalderweireldt L, Winkler G, Forget-Lacoursière EL, Mingelbier M, Siroisa P. 2020. Habitat use by early life stages of the re-established striped bass and conspecific fish species along the St. Lawrence estuary. *Estuarine, Coastal and Shelf Science* 237.

van Deventer H, van Niekerk L, Adams J, Dinala MK, Gangat R, Stephen J Lamberth SJ, Mervyn Lötter M, Namhla Mbona N, Fiona MacKay, Nel1 JL, Ramjukadh CL, Skowno A, Weerts SP. 2020. National Wetland Map 5 – An improved spatial extent and representation of inland aquatic and estuarine ecosystems in South Africa. *Water SA* 46(1).

van Niekerk L, Adams AB, Lamberth SJ, Taljaard S, MacKay CF, Bachoo S, Parak O, Murison G, Weerts SP. 2019a. *Chapter 6: Pressures on the estuarine realm.* South African National Biodiversity Assessment 2018: Technical Report. Volume 3: Estuarine Realm.

van Niekerk L, Lamberth SJ, James N, Taljaard S, Theron A, Krug M. 2019b. *Chapter 10: How vulnerable are South African estuaries to climate change?* South African National Biodiversity Assessment 2018: Technical Report 3.

van Niekerk L, Adams JB, James NC, Lamberth SJ, MacKay CF, Turpie JK, Rajkaran A, Weerts SP, Whitfield AK. 2020. An Estuary Ecosystem Classification that encompasses biogeography and a high diversity of types in support of protection and management. *African Journal of Aquatic Science* 45:1-2, 199-216.

Wasserman J, Claassens L, Adams JB. 2020. Mapping subtidal estuarine habitats with a remotely operated underwater vehicle (ROV). *African Journal of Marine Science* 42(1): 123-12.

Wasserman J, Adams J, Human L. 2022. *Chapter 6: The Role of Plants and Algae in the Functioning of the Knysna Estuary*. Unpublished report.

Watling RJ, Watling HR. 1983. Metal surveys in South African estuaries. VII. Bushmans, Kariega, Kowie and Great Fish Rivers. *Water SA* 9: 66–70.

Whitfield AK. 1984. The effects of prolonged aquatic macrophyte senescence on the biology of the dominant fish species in a southern African coastal lake. *Estuarine, Coastal and Shelf Science* 18: 315–329.

Whitfield AK. 1998. *Biology and ecology of fishes in southern Africa estuaries (Vol. 2)*. Grahamstown: Ichthyological Monographs of the J.L.B. Smith Institute of Ichthyology.

Whitfield AK. 2000. Available scientific information on individual South African estuarine systems. *WATER RESEARCH COMMISSION.*

Whitfield A. 2017. The role of seagrass meadows, mangrove forests, salt marshes, and reed beds as nursery areas and food sources for fishes in estuaries. *Reviews in Fish Biology and Fisheries* 27: 75–110.

Whitfield AK. 2019. Fishes of Southern African Estuaries: From Species to Systems. *Smithiana Monograph* 4:495.

Whitfield AK. 2020. Littoral habitats as major nursery areas for fish species in estuaries: a reinforcement of the reduced predation paradigm. *Marine Ecology Progress Series* 649:219-234.

Whitfield AK, Beckley LE, Bennett BA, Branch GM, Kok HM, Potter IC, van der Elst RP. 1989. Composition, species richness and similarity of ichthyofaunas in eelgrass *Zostera Capensis* bed of southern Africa. *South African Journal of Marine Science* 8:251-259.

Whitfield AK, Kok HM. 1992. Recruitment of juvenile marine fishes into permanently open and seasonally open estuarine systems on the southern coast of South Africa. *Ichthyological Bulletin of the J.L.B Smith Institute of Ichthyology* 57:1-39.

Whitfield AK, Cowley PD. 2010. The status of fish conservation in South African estuaries. *Journal of Fish Biology* 76: 2067-2089.

Whitfield AK, Baliwe NG. 2013. *A century of science in South African estuaries: biology and review of research trends*. South African Network for Coastal and Oceanic Research Occasional Report 7:1-289.

Whitfield AK, Pattrick P. 2015. Habitat type and nursery function for coastal marine fish species, with emphasis on the Eastern Cape region, South Africa. *Estuarine, Coastal and Shelf Science* 160: 49-59

Whitfield AK, Claassens L, Smith K. 2021. *Chapter 8: Fishes of the Knysna Estuary*. Unpublished report.

Winkler G, Dodson JJ, Normand B, Thivierge D, Vincent WF. 2003. Trophic coupling across the St. Lawrence River estuarine transition zone. *Marine Ecology Progress Series* 251:59-73.

APPENDIX

Appendix 1: Literature review of previous ichthyofaunal studies conducted on the Knysna Estuary indicating the year sampling took place, the sampled estuarine region, habitat, season, and gear, and details of fish species caught, including number, length, and estuarine classification.

	Study	**Project aim**	**Estuary Region sampled (refer to Chapter 2, Figure 2.1)**	**Habitat sampled**	**Season**	**Family**	**Species**	**Estuarine Category (as per Whitfield et al. 2019 – see Table 1)**	**Total number of individuals**	**Fish length (mean, range)**	**Gear type**
1	Pollard et al. 2018(data collected in October and November 2014, January and February 2015, April and May 2015 and July and August 2015)	Determine the effect of macroalgal blooms (within eelgrass beds) on the fish assemblages.	Secondary channel in the marine bay (Lower reaches)	Eelgrass (Zostera capensis) beds	Spring 2014 Summer Autumn Winter 2015	*Mugilidae* *Sparidae*					Underwater video footage (Go Pro)

2	Pollard et al. 2017 (data collected monthly between July 1978 and December 1980)	Determine differences between composition of Mugilidae and Sparidae species between Eelgrass and bare substrate.	Throughout the estuary	Eelgrass beds & Bare sediment	Year round				Bare sediment*	Sparse *Zostera**	Dense *Zostera* *		Seine net
						Mugilidae	*Liza richardsonii*	c	0	16.09	9.63	30-39mm	
							Liza dumerili	d	0	7.12	6.8	30-39mm	
							Mugil cephalus	c					
							Myxus capensis	c					
						Sparidae	*Rhabdosargus holubi*	c	53.88	42.55	43.15	90-99mm	
							Lithognathus lithognathus	c	37.14	5.85	9.05		
							Diplodus capensis	b	0	15.99	11.78	20-29mm	
							Sarpa salpa	b	0	8.11	11.52	40-49mm	
							Diplodus hottentotus	c					
3	James and Harrison 2008 (data collected in 1994)	A survey of the ichthyofaunal and physico-chemical characteristics of the estuaries on the South Coast of South Africa.	Throughout the estuary	Shallow banks	Late spring	*Sparidae*	*Rhabdosargus holubi*	c	358				Seine net Gill nets
							Sarpa salpa	b	181				
							Diplodus sargus	b	±110				
							Rhabdosargus globiceps	c	42				
							Lithognathus	c	29				
						Mugilidae	*Liza richardsonii*	c	276				
							juvenile mugilids	c & d	±161				
							Liza dumerili	d	50				
						Clupeidae	*Gilchristella aestuaria*	d	524				

						Atherinidae	*Atherina breviceps*	f	561		
						Haemulidae	*Pomadasys commersonnii*	c	26		
						Gobiidae	*Caffrogobius natalensis*	b	47		
							Psammogobius knysnaensis	I	45		
						Hemiramphidae	*Hemiramphus far*	d	28		
4	de Villiers et al. 2019	Investigation of density and size structures of populations of the endangered Knysna seahorse (Hippocampus capensis)	Secondary channel	Shallow water	Year round	*Syngnathidae*	*Hippocampus capensis*	d	455		Scoop net
5	DFFE sample set (collected from 2007 – 2012)	Unpublished data from DFFE's sampling within the Knysna estuary	Throughout the estuary		Summer and winter samples	*Mugilidae*	*Chelon richardsonii*	c	6487		Seine net
							Mugil cephalus	c	15		
							Mugilidae (small mullets)	d	2860		
							Chelon dumerili	d	520		
							Chelon tricuspidens	d	215		

							Pseudomyxus capensis	f	40		
						Sparidae	*Rhabdosargus holubi*	c	5022		
							Diplodus sargus	b	703		
							Lithognathus lithognathus	c	192		
							Rhabdosargus globiceps	c	341		
							Lithognathus mormyrus	c	3		
							Sarpa salpa	b	321		
							Spondyliosoma emarginatum	c	111		
							Cheimerius nufar	a	62		
							Diplodus cervinus	b	4		
						Carangidae	*Lichia amia*	c	70		
							Trachurus capensis	a	136		
							Caranx heberi	a	1		
						Clupeidae	*Gilchristella aestuaria*	c	5719		
						Atherinidae	*Atherina breviceps*	f	83585		
						Gobiidae	*Psammogobius knysnaensis*	d	2694		

							Redigobius dewaalii	j	26		
							Caffrogobius spp.	d	2359		
							Glossogobius callidus	f	17		
						Haemulidae	*Pomadasys commersonnii*	c	109		
							Pomadasys olivaceum	c	150		
						Syngnathidae	*Syngnathus temminckii*	c	511		
						Soleidae	*Solea turbynei*	b	168		
							Heteromycteris capensis	c	756		
						Clinidae	*Clinus superciliosus*	e	1384		
						Hemiramphidae	*Hyporhamphus capensis*	c	550		
							Hemiramphus far	b	42		
						Tetraodontidae	*Amblyrhynchotes honckenii*	c	225		
						Triglidae	*Cheilidonichthys capensis*	b	1		
						Monodactylidae	*Monodactylus falciformis*	c	401		
						Pomatomidae	*Pomatomus saltatrix*	b	87		

						Syngnathidae	*Hippocampus capensis*	d	77		
						Dasyatidae	*Dasyatis chrysonota*	a	4		
						Blennidae	*Omobranchus woodi*	c	1		
						Clupeidae	*Sardinops sagax*	a	23		
						Chaetodontidae	*Chaetodon marleyi*	b	4		
						Gymnuridae	*Gymnura natalensis*	a	3		
						Engraulidae	*Engraulis japonicus*	b	3		
						Gobiesocidae	*Apletodon pellegrini*	b	14		
						Fistulariidae	*Fistularia commersonii*	a	1		
						Ostraciidae	*Ostracion cubicus*	c	1		
						Ariddae	*Galeichtyhys feliceps*	b	20		
						Sciaenidae	*Argyrosomus japonicus*	c	3		
						Engraulidae	*Engraulis japonicus*	a	3		
						Dussumieriidae	*Etrumeus whiteheadi*	b	2		
						Myliobatidae	*Myliobatis aquila*	e	2		

							Aetomylaeus bovinus	a	1		
						Centrarchidae	*Lepomis macrochirus*	i	1		
						Odontaspididae	*Carcharias taurus*	a			
6	Grindley. 1985.	A list of common fish species found in the Knysna Estuary				*Scyliorhinidae*	*Poroderma africanum*	a			
						Rhinobatidae	*Acroteriobatus annulatus*	a			
						Myliobatidae	*Myliobatis aquila*	b			
						Dasyatidae	*Dasyatis pastinaca*	b			
						Gymnuridae	*Gymnura natalensis*	a			
						Torpedinidae	*Torpedo fuscomaculata*	c			
						Elopidae	*Elops machnata*	b			
						Clupeidae	*Gilchristella aestuaria*	c			
							Sardinops ocellatus	a			
						Engraulidae.	*Engraulis capensis*	a			
						Ariidae	*Galeichthys feliceps*	b			
						Hemiramphidae	*Hyporhamphus knysnaensis*	b			

							Hemiramphus far	b			
						Synodontidae	*Trachinocephalus myops*	c			
						Soleidae	*Synapturichthys kleinii*	b			
						Soleidae	*Heteromycterus capensis*				
							Solea bleekeri	b			
						Syngnathidae	*Hippocampus capensis*	d			
							Syngnathus acus	c			
							Hippichthys spicifer	c			
						Terapontidae	*Terapon jarbua*	c			
						Serranidae	*Serranus cabrilla*	a			
						Carangidae	*Lichia amia*	c			
						Pomatomidae	*Pomatomus saltatrix*	b			
						Sciaenidae	*Argyrosomus japonicus*	c			
							Umbrina canariensis	a			
						Monodactylidae	*Monodactylus falciformis*	c			
						Chaetodontidae	*Chaetodon marleyi*	b			
						Dichistiidae	*Dichistius capensis*	a			

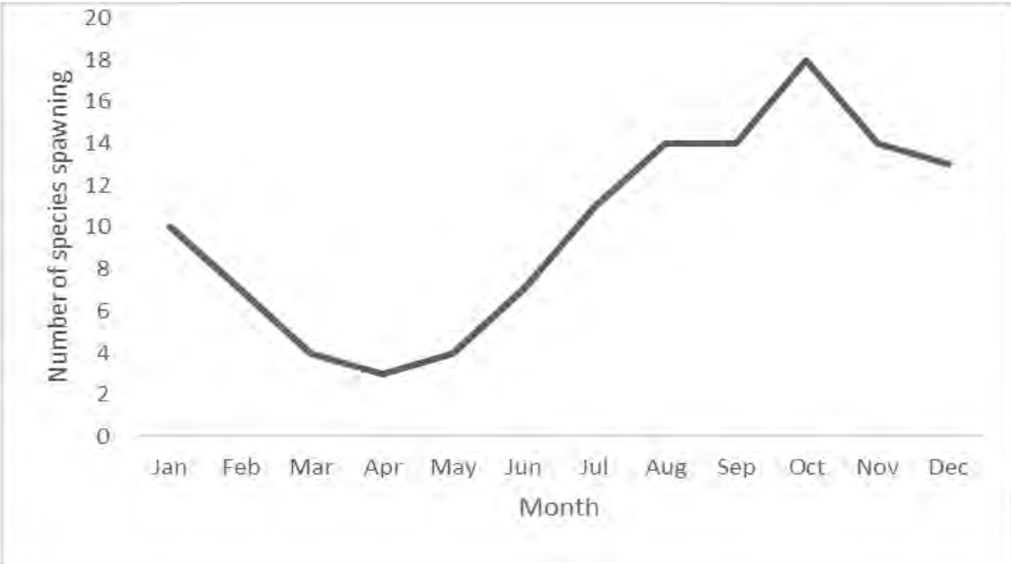
						Haemulidae	*Pomadasys olivaceus*	c			
							Pomadasys commersonnii	c			
						Sparidae	*Rhabdosargus holubi*	c			
							Diplodus sargus	b			
							Lithognathus lithognathus	c			
							Rhabdosargus globiceps	c			
							Lithognathus mormyrus	c			
							Sarpa salpa	b			
							Spondyliosoma emarginatum	c			
							Diplodus cervinus	b			
							Rhabdosargus sarba	c			
						Mugilidae	*Liza richardsonii*	c			
							Mugil cephalus	c			
							Liza dumerili	c			
							Liza tricuspidens	d			
							Myxus capensis	f			
						Atherinidae	*Atherina breviceps*	d			

						Gobiidae	*Psammogobius knysnaensis*	d			
							Redigobius dewaali	j			
							Caffrogobius nudiceps	d			
						Blenniidae	*Omobranchus woodi*	d			
						Clinidae	*Clinus superciliosus*	b			
						Platycephalidae	*Platycephalus indicus*	c			
						Triglidae	*Chelidonichthys kumu*	c			
						Gobiesocidae	*Apletodon pellegrini*	b			
						Anguillidae	*Anguilla mossambica*	h			
						Ophichthidae	*Ophisurus serpens*	b			
						Congridae	*Conger wilsoni*	b			
						Diodontidae	*Diodon hystrix*	c			
						Tetraodontidae	*Lagocephalus laevigatus*	b			
7	Claassens and Harasti. 2020.	Life history and populations dynamics of	Secondary channel	Shallow bank with	February 2018-April 2019	*Syngnathidae*	*Hippocampus capensis*	d	78		Scoop net

		Knysna Seahorse		Reno mattress								

Species/Common name	Spawning seasons	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Estuarine Guild
Fistularia commersonii/ Smooth Flutemouth														M
Chrysoblephus laticeps/ Red Roman	Oct – Jan													M
Cymatoceps nasutus/ Black musselcracker	May – Oct (Transkei waters)													M
Polysteganus praeorbitalis /Scotsman	Winter and Spring													M
Sparodon durbanensis/ White musselcrakcer	Aug – Jan													M
Cheimerius nufar/ Santer	Nov – Feb (Peaks Dec – Jan)													M
Chelon richardsonii/ Southern Mullet	Early and late summer peaks													MED
Mugil cephalus/ Flathead mullet	Autumn and spring (extended spawning season)													MED
Clinus superciliosus/ Super klipvis	Winter – Early summer													MED
Rhabdosargus holubi/ Cape Stumpnose	Jul – Feb (Peak Aug – Dec)													MED
Lithognathus lithognathus/ White Steenbras	Jul – Aug (late winter)													MED
Hyporhamphus capensis/ Cape halfbeak	Oct – Mar													MED
Spondyliosoma emarginatum/ Steenjie	Jul – Oct													MED
Heteromycteris capensis/ Cape sole	Peaks Oct – Feb													MED
Pomadasys olivaceum/ Piggy	Mar – Dec													MED
Stolephorus holodon/ Thorny anchovy														MED
Lichia amia/ Leervis	Sep – Nov (KZN Waters)													MED

Monodactylus falciformis/ Oval moony	Oct – Feb		MED
Amblyrhynchotes honckenii/ Evileye blaasop	End of winter		MED
Argyrosomus japonicus/ Dusky kob	Oct – Jan		MED
Diplodus sargus capensis/ Blacktail	Aug – Mar (Sep – Dec peaks)		MEO
Sarpa salpa/ Strepie	Apr – Sep (winter and spring)		MEO
Diplodus cervinus hottentotus/ Zebra	Aug – Dec (peaking in Oct)		MEO
Solea bleekeri/ Blackhand sole	June – Dec		MEO



Appendix 2: Diagrammatic representation of a review of spawning seasons of potential demersal sample species within the Knysna Estuary, along with nearshore demersal marine fisheries species, in order to identify recruitment sampling periods. Species spawning periods were defined as per Mann 2013. Species estuarine guild categorised as per Whitfield 2019: marine straggler = MS, marine-estuarine dependent = MED, marine-estuarine opportunist = MEO, including the total number species spawning each month.