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Occurrence of mugilid and sparid fishes in *Zostera capensis* and bare sediment habitats of the Knysna Estuary

by

MELISSA POLLARD

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Supervisor: Prof. A.N. Hodgson
Co-supervisor: Prof. A.K. Whitfield

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Abstract

Seagrass meadows are regarded as one of the most unique and valuable ecosystems in the biosphere, primarily because of the variety of services that they provide. Seagrass meadows serve as nursery grounds for many species and often play an important role in the juvenile stages of economically and recreationally important fish. *Zostera capensis* Setchell is one of the most dominant submerged macrophytes and the most common seagrass in South African estuaries and is often referred to as eelgrass. *Zostera capensis* meadows occupy a large area within the Knysna Estuary but little is known about their importance to associated fish assemblages. With *Z. capensis* meadows being under increased pressure from anthropogenic influences, it is important to establish which fish species and families utilize these habitats and what role they play in the ecology of fish assemblages within the Knysna Estuary. The primary objective of this study was to compare the use of *Z. capensis* and adjacent bare sediment areas by mainly large juvenile and subadult sparids and mugilids using different techniques. The two main hypotheses were as follows; 1) Mugilidae are likely to be more dominant in the unvegetated areas of the estuary littoral and Sparidae are likely to predominate within the *Z. capensis* bed areas of the estuary littoral. 2) The non-destructive underwater video monitoring method would yield similar fish composition data to seine netting sampling of the identical sites. Both hypotheses were assessed using data collected during this study and the analysis of historical unpublished data. Overall, Mugilidae were more abundant at bare unvegetated areas where they did most of their foraging. Sparidae were more abundant in the *Z. capensis* beds, which was also the habitat where they primarily foraged. With regards to the comparison of two different sampling methods, namely underwater video monitoring and seine netting, similar patterns arose with regards to the fish species observed in camera footage and those captured in the seine net, although the abundances were not always comparable.

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Chapter 1 - General Introduction

1.1 Seagrass ecology

Seagrasses, a group of aquatic flowering plants that have adapted to be fully submerged in salt water, have successfully colonized all the seas except the most polar (Orth *et al.*, 2006). These macrophytes have evolved distinctive morphological, ecological and physiological adaptations for their complete submergence, including epidermal chloroplasts, internal gas transport, marine dispersal and submarine pollination (Short and Wyllie-Echeverria, 1996; Orth *et al.*, 2006). Seagrasses require high light levels as they often grow in highly reduced sediments with toxic sulphide levels and need to supply oxygen to their rhizomes and roots, and also have to support a relatively large proportion of non-photosynthetic tissue (Orth *et al.*, 2006). Duarte (1991) studied the depth limit of seagrasses and found that seagrasses could grow from mean sea level down to a depth of 90 meters. Later studies by Short *et al.* (2007) showed that different oceanic areas showed different seagrass depth limits. Seagrasses in the temperate North Atlantic have the shallowest maximum depth at 12 meters and the tropical Indo-Pacific the deepest maximum depth at 70 meters while the temperate North Pacific (20 m), tropical Atlantic (50 m), Mediterranean (50 m) and Southern Oceans (50 m) lie between these two depths. These plants are found in marine and estuarine environments over a wide range of latitudes in both the southern (as far down as 47°16′36.70″ S) and northern (as far up as 66°23′40.05″ N) hemispheres (Short and Wyllie-Echeverria, 1996; Short *et al.*, 2007).

Seagrass beds have important functions in shallow marine environments (Beck *et al.*, 2001; Duarte, 2002) where they greatly influence biological, physical and chemical environments (Orth *et al.*, 2006). They act as ecological engineers and are recognized as a key component in many coastal zone ecosystems (Duarte, 2002; Becker *et al.*, 2012), primarily due to their high primary and associated secondary productivity (Beck *et al.*, 2001) which rivals that of many of the most productive biomes on earth (Short and Wyllie-Echeverria, 1996; Orth *et al.*, 2006). The global extent of seagrasses is estimated to be between 177 000 and 600 000 km^2 (Duarte, 2002; McLeod *et al.*, 2011; Fourqurean *et al.*, 2012), covering less than 0.2% of the world's ocean area (Fourqurean *et al.*, 2012). Although seagrasses occupy such a small percentage of the globe, the total carbon burial is estimated to be between 48 and 112 teragrams C yr^{-1} for this habitat (McLeod *et al.*, 2011; Fourqurean *et al.*, 2012). Seagrass beds, therefore, are responsible for approximately 15% of the carbon storage in the ocean (Duarte, 2002) and it is estimated that 50% of the carbon sequestered in these habitat sediments is of external origin (McLeod *et al.*, 2011). Seagrass beds play an important role in terms of trophic links with other ecosystems, exporting approximately 24%, on average, of their net production to adjacent ecosystems (Duarte, 2002).

Seagrass meadows are regarded as one of the most unique and valuable ecosystems in the biosphere, primarily because of the variety of services that they provide (Duarte, 2002; Jackson *et al.*, 2006; Orth *et al.*, 2006; Adams, 2016). Some of these services include; organic carbon export to adjacent ecosystems, wave attenuation, trapping and cycling of nutrients, provision of food for coastal food webs, prevention of sediment resuspension, habitat for microbes as well as invertebrates and vertebrates, provision of oxygen to littoral waters and sediments, sediment stabilization, shoreline protection and carbon sequestration from the atmosphere (Whitfield *et al.*, 1989; Sogard and Able, 1991; Guidetti, 2000; Duarte, 2002; Orth *et al.*, 2006; Adams, 2016).

Seagrass meadows can act as biological sentinels or indicators since changes in seagrass distribution and abundance (e.g. widespread seagrass loss or reduction in the maximum depth limit) signal key losses of ecosystem services that these environments provide. Seagrasses are also valuable biological indicators as they integrate environmental impacts over definable and measurable time scales (Orth *et al.*, 2006).

1.2 Anthropogenic impacts on seagrasses

Estuaries have been described as one of the most degraded aquatic environments on the planet, mainly because coastal areas (and estuaries in particular) have been key areas for human colonization for hundreds of years (Beck *et al*., 2001). The growing human population and demand for food has caused the overexploitation of resources associated with seagrass beds, especially fish communities (Pogoreutz *et al*., 2012). Although seagrasses are distributed worldwide, they have a relatively low taxonomic diversity when compared with other macrophyte taxonomic groups with the same kind of distribution, which makes them particularly vulnerable to human disturbances as they are not descended from multiple and diverse evolutionary lineages such as mangroves, salt marsh plants and marine algae (Orth *et al*., 2006).

Unfortunately seagrasses are experiencing a worldwide decline and are listed as Vulnerable by the IUCN (Short *et al*., 2010; Adams, 2016) owing to anthropogenic disturbances such as deterioration in water quality and direct physical damage (Short and Wyllie-Echeverria, 1996; Duarte, 2002; Orth *et al*., 2006; Adams, 2016). Seagrasses occur in shallow, protected coastal waters and are thus in the direct path of sediment inputs and watershed nutrients that makes them highly susceptible to these inputs (Short and Wyllie-Echeverria, 1996; Jackson *et al*., 2006; Orth *et al*., 2006). As they have high photosynthetic active radiation requirements, seagrasses are highly responsive to environmental changes, particularly those that alter water clarity (Orth *et al*., 2006).

Anthropogenic impacts on seagrass beds can be divided into two major categories, proximal and indirect impacts. Proximal impacts that affect seagrass meadows locally include coastal engineering and aquaculture, siltation, eutrophication, mechanical damage and damage from maintenance and construction of infrastructures within the coastal zone (Dibble *et al*., 1996; Short and Wyllie-Echeverria, 1996; Duarte, 2002; Hughes *et al*., 2002; Jackson *et al*., 2006; Orth *et al*., 2006). Indirect impacts affect seagrass meadows far away from the area of disturbance, and include global anthropogenic changes such as the large-scale modification of food webs through fisheries, sea-level rise, global warming and CO_2 and UV increases (Duarte, 2002; Jackson *et al.*, 2006; Orth *et al.* 2006). One of the most unmistakeable anthropogenic impacts on seagrass meadows is physical disturbance within the coastal zone associated with increasing services for the growing coastal populations and increasing number of ports that are being constructed or expanded (Short and Wyllie-Echeverria, 1996; Orth *et al.*, 2006).

Coastal developments cause many problems, including changes in sediment transport, deposition, and reductions in water transparency (Duarte, 2002), with the latter impact being the most significant and common cause in the decline of seagrass habitats (Short and Wyllie-Echeverria, 1996; Orth *et al.*, 2006). When these problems are combined with the influence of other organisms such as algal blooms and brown 'tides', the trajectory of seagrass loss is accelerated (Orth *et al.*, 2006). Fisheries operations also cause damage to seagrass meadows, particularly where shallow trawling is practiced, as well as smaller scale activities associated with fishing, e.g. use of push nets over shallow areas and digging for bait in the intertidal and subtidal zone (Short and Wyllie-Echeverria, 1996; Duarte, 2002; Hughes *et al.*, 2002; Mucina *et al.*, 2006; Orth *et al.*, 2006). The increasing human population associated with coastal zones will ultimately increase the physical disturbance of seagrass meadows and also increase the nutrient and sewage discharge to coastal waters, thereby expanding and intensifying the already widespread eutrophication problems (Duarte, 2002; Hughes *et al.*, 2002; Orth *et al.*, 2006).

Seagrasses have been shown to be influenced by both bottom-up and top-down processes as the decrease of higher-level consumers in seagrass meadows has been shown to have a cascading effect on the trophic dynamics within such systems (Orth *et al.*, 2006; Adams, 2016). The loss of seagrass meadows results in a loss of all the associated functions and services that seagrasses supply to coastal zones (Duarte, 2002). This involves a decrease in overall littoral primary production and the loss of sediment protection provided by seagrass canopies, thus increasing the deterioration in photosynthetic conditions for remaining seagrass plants (Duarte, 2002; Sheppard *et al.*, 2011). There is also a shift in the coastal ecosystem due to a reduction in the variety of primary producers and the proliferation of anoxic sediment conditions because of a loss of sediment oxygenation provided by seagrass roots (Duarte, 2002).

"Wasting disease" has been attributed to the reduction in distribution and productivity of seagrasses in estuaries and bays, and can affect entire coastlines, e.g. as occurred in the 1930s where it greatly affected Atlantic and Pacific populations of seagrass, including Europe, Canada and the United States (Short and Wyllie-Echeverria, 1996). The 1930's outbreak of *Zostera* wasting disease in Denmark resulted in a decline, in some instances disappearance, of many invertebrate and fish species, some of which showed no apparent trophic links to the *Zostera* beds (Pollard, 1984). In some areas, the *Zostera* populations never recovered from the wasting disease, including New Jersey where the *Zostera marina* meadows were replaced by *Ulva* spp. and *Enteromorpha* spp. algal beds (Sogard and Able, 1991).

The slime mould *Labyrinthula zosterae* has been found to infect various *Zostera* spp. and is the causative agent for the above "wasting disease" as it damages the leaf material and eventually makes it non-functional (Short and Wyllie-Echeverria, 1996). *Labyrinthula* spp. occur in both hemispheres and has also been linked to major *Zostera* spp. die-off in New Zealand, Japan, the Virgin Islands, and the northern Gulf of Mexico (Short and Wyllie-Echeverria, 1996).

In summary, the loss of seagrass meadows has been shown to lead to modifications in coastal food webs, a decrease in fish production, decline in fish species richness, change in the relative abundance of all species and the loss of harvestable resources from the ecosystem (Duarte, 2002; Sheppard *et al.*, 2011). Thus the demise of seagrasses represents a major loss in economic terms, as well as a decline in the ecological and biodiversity value of such littoral habitats to the coastal ecosystem as a whole (Duarte, 2002).

1.3 South African seagrass beds and associated fishes

Zostera capensis Setchell is one of the most dominant submerged macrophytes (Adams and Bate, 1994a; Barnes, 2013) and the most common seagrass in South African estuaries (Adams and Talbot, 1992; Adams, 2016), occurring in 62 of the approximately 300 estuaries (Adams, 2016) and is often referred to as Cape dwarf-eelgrass or simply eelgrass (Barnes, 2010a; Barnes and Ellwood, 2012; Adams, 2016). *Zostera capensis* meadows occur in sheltered bays and estuaries along the southern African coast from Inhaca Island (Edgcumbe 1980) and Maputo Bay (Mozambique) (Adams, 2016) in the north-east to Langebaan Lagoon (Edgcumbe 1980) and Olifants Estuary (Adams, 2016) in the south-west. *Zostera capensis* is a low intertidal species and beds are usually restricted to the lower and middle reaches of permanently open estuaries but can be found in the upper reaches of systems deprived of fresh water (Howard-Williams and Liptrot, 1980; Ter Morshuizen and Whitfield, 1994; Bornman *et al.*, 2008; Adams, 2016) as well as some systems that are periodically closed off from the sea (Whitfield *et al.*, 1989). In systems that are dominated by freshwater inputs, however, *Z. capensis* is usually absent (Adams and Talbot, 1992; Adams and Bate, 1994a; Mucina *et al.*, 2006).

Due to its flexible leaves, strong root system and protected meristem, *Z. capensis* is able to endure periods of desiccation and exposure and can also grow in areas with strong tidal currents (Adams, 2016). This species is seen to demonstrate great morphological plasticity as it has narrower and shorter leaves at sites where more exposure occurs and broader and longer leaves at sites with calmer and deeper water (Adams, 2016). Adams (2016) states that the biomass of *Z. capensis* is aseasonal and changes in response to changes in physico-chemical conditions and episodic flood events. After these flood events, *Z. capensis* populations can take between one and three years to recover (Adams, 2016). *Zostera capensis* is listed as Vulnerable by the IUCN as it occupies an area less than 2000 km^2 (Short *et al.*, 2010; Adams, 2016). The populations are severely fragmented and there is strong evidence that the total southern African estuarine area under *Z. capensis* is decreasing (Short *et al.*, 2010; Adams, 2016).

Millions of larval and juvenile marine fishes enter southern African estuaries during late winter, spring and early summer each year, with these environments being utilised as sheltered and food-rich nursery areas favouring rapid growth (Wallace *et al.*, 1984; Whitfield, 1994). Beck *et al.* (2001) identified nurseries as areas where invertebrates or juvenile fish species occur at higher densities, grow faster, or avoid predation more effectively than in other habitats. Any environment that has a greater than average input in the recruitment of juveniles into adults can be seen as a nursery area. Whitfield and Pattrick (2015) give four criteria that a habitat should offer to serve as a nursery for fish including; physiological suitability in terms of physical and chemical features, connectivity that enables colonisation by juveniles or larvae, provision of copious and suitable food sources and protection from predators.

Wallace *et al.* (1984) found that 81 species of fish are partially or wholly dependent on South African estuaries and Weerts and Cyrus (2002) showed that at least 66% of the fishes in southern African estuaries are partially dependent on estuaries for their existence. Whitfield (1998) documented some of the fish that use estuaries as nursery areas (Table 1.1). These fish can be divided into two main groups, the first comprising of euryhaline marine species that spawn out at sea and make extensive use of estuarine environments during juvenile and/or adult life stages and are known as marine species (Whitfield, 1998). The second group spawn within estuaries, are relatively few in number, invariably small, and are known as estuarine species (Whitfield, 1998).

Seagrasses exhibit considerable habitat complexity due to their structure which increases the volume and surface area for food production and shelter that is available to newly-settled fish larvae when compared to other habitat types (Bennet and Branch, 1990; Dibble *et al.*, 1996; Guidetti, 2000; Beck *et al.*, 2001; Pogoreutz *et al.*, 2012). Faunal densities in South Africa and elsewhere are often orders of magnitude higher inside seagrass meadows compared with adjacent unvegetated areas (Whitfield, 1988; Sogard and Able, 1991; Hughes *et al.*, 2002), with a greater diversity, abundance and biomass of fish and larger number of juveniles found in vegetated areas compared with bare unvegetated habitats (Heck *et al.*, 1989; Guidetti, 2000; Hughes *et al.*, 2002; Pogoreutz *et al.*, 2012). Barnes and Barnes (2014), however, found that in the Knysna Estuary invertebrate species density was higher by only 1.13 to 1 in *Z. capensis* beds compared with unvegetated areas and invertebrate abundance was in fact lower in *Z. capensis* with a ratio of 0.64 to 1 compared with unvegetated areas. They proposed that the differences in invertebrate species density and abundance might not be as a result of *Z. capensis* being a more favourable habitat but that the processes in operation within the unvegetated sediments might also be playing a role through bioturbation of the sediments (Barnes and Barnes, 2014). Paterson and Whitfield (2000) in their study of 0+ juvenile fishes in the Kariega Estuary salt marshes, showed that shallow estuarine habitats, regardless of the vegetation or lack thereof, provided a refuge for juvenile fishes through low predation levels in these shallow areas. Therefore, this study focused on both *Z. capensis* beds and bare, unvegetated areas as both of these shallow littoral habitats are known to provide a nursery area for juvenile fish.

Seagrass meadows serve as nursery grounds for many species (Bell *et al.*, 1988; Guidetti, 2000; Beck *et al.*, 2001) and often play an important role in the juvenile stages of economically and recreationally important species of shellfish and finfish (Beckley, 1983; Hanekom and Baird, 1984; Bennet and Branch, 1990; Sogard and Able, 1991; Ter Morshuizen and Whitfield, 1994). They may also be important as part of the range of organic olfactory cues that help the larvae of some marine fishes locate estuaries and then recruit into them (James, 2007; Sheppard *et al.*, 2011). In this regard, it is perhaps significant that Dibble *et al.* (1996) found that smaller and younger fishes become more abundant as submerged plant density increased.

Table 1.1: Some of the marine and estuarine fish species known to use estuarine environments as nursery areas. Modified from Whitfield (1998).

Family	Species	Marine/Estuarine
Ambassidae	*Ambassis natalensis*	Estuarine
	Ambassis productus	Estuarine
Ariidae	*Galeichthys feliceps*	Marine
Atherinidae	*Atherina breviceps*	Estuarine
Carangidae	*Caranx ignobilis*	Marine
	Caranx sexfasciatus	Marine
	Lichia amia	Marine
Chanidae	*Chanos chanos*	Marine
Clupeidae	*Gilchristella aestuaria*	Estuarine
	Hilsa kelee	Marine
Clinidae	*Clinus spatulatus*	Estuarine
	Clinus superciliosus	Estuarine
Gerreidae	*Gerres acinaces*	Marine
	Gerres methueni	Marine
Gobiidae	*Caffrogobius gilchristi*	Estuarine
	Caffrogobius nudiceps	Estuarine
	Glossogobius callidus	Estuarine
	Croilia mossambica	Estuarine
	Periophthalmus argentilineatus	Estuarine
	Psammogobius knysnaensis	Estuarine
Haemulidae	*Pomadasys commersonii*	Marine
	Pomadasys olivaceum	Marine
Hemiramphidae	*Hyporhamphus capensis*	Estuarine
Leiognathidae	*Leiognathus equula*	Marine
Lutjanidae	*Lutjanus argentimaculatus*	Marine
	Lutjanus fulviflamma	Marine
Monodactylidae	*Monodactylus argenteus*	Marine
	Monodactylus falciformis	Marine
Mugilidae	*Liza dumerili*	Marine
	Liza macrolepis	Marine
	Liza richardsonii	Marine
	Liza tricuspidens	Marine
	Mugil cephalus	Marine
	Myxus capensis	Marine
	Valamugil cennesius	Marine
	Valamugil buchanani	Marine
Platycephalidae	*Platycephalus indicus*	Marine
Pomatomidae	*Pomatomus saltatrix*	Marine
Sciaenidae	*Argyrosomus japonicas*	Marine
	Johnius dorsalis	Marine
	Otolithes ruber	Marine
Sparidae	*Acanthopagrus berda*	Marine
	Diplodus capensis	Marine
	Lithognathus lithognathus	Marine
	Rhabdosargus globiseps	Marine
	Rhabdosargus holubi	Marine
	Rhabdosargus sarba	Marine
	Sarpa salpa	Marine
Sphyraenidae	*Sphyraena barracuda*	Marine
Syngnathidae	*Hippocampus capensis*	Estuarine
	Syngnathus temminckii	Estuarine
	Syngnathus watermeyeri	Estuarine
Terapontidae	*Terapon jarbua*	Marine

Whitfield *et al.* (1989) reviewed the ichthyofauna found in *Z. capensis* beds of eight South African estuaries (Richards Bay, Mngazana, Bushmans, Sundays, Swartkops, Kromme, Knysna and Swartvlei estuaries) and one coastal lagoon (Langebaan Lagoon). In addition, Ter Morshuizen and Whitfield (1994) and Paterson and Whitfield (2000) examined the ichthyofauna associated with *Z. capensis* beds in the Kariega Estuary. In these 10 systems a total of 100 fish species from 44 families were associated with *Z. capensis* (Appendix 1). Of these 44 families, the Sparidae were the biggest group constituting 12% of the species, with Gobiidae the second largest group at 9%, and Mugilidae third with 8%. One species of Sparidae, *Diplodus capensis*, was found in all 10 systems while *Rhabdosargus holubi* was found in all the systems except the cool temperate Langebaan Lagoon (Appendix 1). Other species were recorded in only one system, for example *Lithognathus mormyrus* was only recorded in the Knynsa Estuary and *Pagellus natalensis* was only recorded in the Swartvlei Estuary (Appendix 1). This information highlights that the Sparidae are the most diverse family with regards to the species associated with South African *Z. capensis* habitats, which led to one of the hypotheses given in Section 1.5. Although Mugilidae are often found associated with *Z. capensis* beds (Appendix 1), this fish family is known to be mainly detritivorous with a high proportion of microphytobenthos in their diet. Benthic diatoms are readily available on bare sediments and it is hypothesized that Mugilidae would be more abundant in bare, unvegetated areas than in *Z. capensis* beds (Beckley, 1983; Hanekom and Baird, 1984; Whitfield, 1988; Whitfield *et al.*, 1989).

1.4 Monitoring techniques for fishes in submerged macrophyte bed

Most of the commonly used sampling techniques for the monitoring and assessment of fishes associated with submerged macrophyte beds in coastal and transitional environments are based on traditional fishing gear, including long lines, trap nets, gill nets, trammel nets, seine nets and trawling (Franco *et al.*, 2011). Drop nets and pop nets can sometimes measure the abundance, distribution and diversity of juvenile and adult fishes in densely vegetated and complex habitats where seining is ineffective (Dibble *et al.*, 1996). In addition, the use of modified ichthyoplankton nets can work well for sampling larval fishes in structurally complex habitats where tow nets are not easy to use (Dibble *et al.*, 1996).

Heck *et al.* (1989) used a 4.9 m otter trawl with 19 mm mesh wings and a 6 mm mesh liner for four to five 2 minute tows over *Zostera capensis* meadows to collect fishes and decapod crustaceans. Jackson *et al.* (2006) used a wide range of sampling techniques to collect fish species over seagrass beds, which included diver surveys, push netting, beach seines, traps and trawls. In the above study they found that a combination of trawling and beach seine sampled the optimum range of target fish species. Ferrell and Bell (1991) used a seine net with a stretched mesh size of 6 mm to collect juveniles of large fish species and small species over *Zostera capricorni* and bare sand areas. In South Africa, three different types of nets were used for sampling fishes in the Swartvlei Estuary, both over *Z. capensis* beds and sites with a mixture of bare sand and filamentous algae, and these included a set of multifilament gill nets, a large monofilament seine net and a small multifilament seine net (Whitfield, 1988). A small seine net, with a stretched mesh size of 2 mm, was used to collect fishes over *Z. capensis* beds in a study focusing on juvenile fishes in the Swartkops Estuary (Beckley, 1983). Hanekom and Baird (1984) used a 10 m seine net with a stretched mesh of 2 mm to collect fish over *Zostera* and non-*Zostera* sites in the Kromme Estuary. A 5 m fry-seine net with a 0.5 mm bar mesh size was used to sample juvenile and small fish species over *Zostera* beds in the Kariega Estuary (Ter Morshuizen and Whitfield, 1994). Bennet and Branch (1990) used a 25 m seine net with a stretched mesh size of 10 mm to collect fish over *Ruppia* and *Potamogeton* beds in the Bot estuary.

In the Sundays Estuary a wide range of submerged macrophytes were present *(Potamogeton crispus, Phragmites australis, Vaucheria* spp. and *Zostera capensis*) and Beckley (1984) opted for two different nets to sample in these vegetated areas, namely a large 50 m x 2 m seine net with 12 mm stretched mesh, and a small 10 m x 2 m seine net with 2 mm stretched mesh. Sheppard *et al.* (2011) used a small mesh seine net (30 m x 2 m with a 5 mm bar) to sample the small estuarine-spawning fishes and a large mesh seine net (50 m x 2 m with a 15 mm bar) for the larger marine-spawning species in *Potamogeton pectinatus* and *Ruppia cirrhosa* beds in the East Kleinemonde Estuary. In the estuarine Swartvlei lake, Whitfield (1986) opted to use gill nets as the main sampling gear because seine and trawl netting through the dense *Potamogeton* bed canopy was found to be impractical.

Rotenone has been used to collect fishes from some submerged macrophyte beds. Vegetated areas are usually blocked off with nets and the pesticide applied; however the efficiency of fish collection decreases as plant density increases (Dibble *et al.*, 1996). An example of such a study is that by Bell *et al.* (1988) using 1 mm mesh block nets to enclose the *Zostera capricorni* beds affected by the rotenone poison. Using this technique they collected 9943 fishes from 78 species from 12 different sampling sites (Bell *et al.*, 1988).

Underwater visual surveys by observers in submersibles in macrophyte beds in deeper marine waters and SCUBA divers in shallow waters have allowed density estimates to be obtained for a variety of fish species. Divers can rapidly census fish populations and measure species composition and abundance in habitats that are difficult to sample using traditional methods (Dibble *et al.*, 1996; Harvey *et al.*, 2007). The drawback of this survey technique is that there is a bias against benthic and cryptic species. In addition, the limited availability of submersibles and depth limitations of SCUBA divers, together with the need for low or moderate submerged plant density and high water clarity, restricts the potential use of these types of surveys (Dibble *et al.*, 1996; Harvey *et al.*, 2007).

Guidetti (2000) opted for the non-destructive diver visual census method to assess the abundance of fishes in different habitats. This technique was regarded as being more appropriate for fast swimming species and smaller fishes, and consisted of swimming along a 20 m long and 2 m wide transect and identifying and counting the fishes within the transect. This method resulted in 34 fish species being recorded in three different habitats at one site and 20 species at another site. Mugilidae, however, were not identified down to species level as there was difficulty in the specific determination by the direct visual observation (Guidetti, 2000). A rapid visual census along belt transects of 25 m in length and 6 m in width was used by Pogoreutz *et al.* (2012) and consisted of snorkelling by a single observer so as to reduce disturbance caused by diver activity (Pogoreutz *et al.*, 2012). This technique showed that inexpensive, non-destructive and rapid data collection was possible and very comparable to the methods used in other more complex ecological fish studies (Pogoreutz *et al.*, 2012).

Acoustic methods have also been used to survey fish such as hydroacoustics which rely on sonar systems (Murphy and Jenkins, 2010). Under the many branches of hydroacoustics, split-beam echosounders, acoustic tagging and acoustic cameras are most often used in the monitoring of fish (Murphy and Jenkins, 2010). Split-beam echosounders look at the biomass of fish aggregations and can estimate absolute population size while being a non-destructive sampling method (Murphy and Jenkins, 2010). The method surveys large areas and equipment is portable and easy to use but there is some taxonomic ambiguity and the equipment cannot detect fish that are less than 2 m off the substratum (Murphy and Jenkins, 2010). Acoustic tagging target larger juvenile and adult fish species that are mobile (Murphy and Jenkins, 2010). This technique can be combined with other observational sampling methods and it supplies data on spatial and temporal movement of multiple individuals without the use of recapture methods (Murphy and Jenkins, 2010). Some of the tags, however, have to be surgically implanted which can reduce the sample size and the fish have to be moving within the range of the receivers (Murphy and Jenkins, 2010). Acoustic cameras such as dual-frequency identification sonars (DIDSON) track the movement and behaviour of fish (Murphy and Jenkins, 2010). These cameras can be used in low-light and turbid waters and give high resolution data on direction of travel of multiple targets (Murphy and Jenkins, 2010). Habitat structure, however, can obstruct the beams as well as producing a low taxonomic resolution and the technique is limited to small-scale studies (Murphy and Jenkins, 2010). The biggest obstacle with these hydroacoustics though are the fact that the equipment is very expensive (Murphy and Jenkins, 2010) and thus does not make it a viable option for most research efforts.

Remotely operated video stations are another non-destructive and non-intrusive method for monitoring fish composition and behaviour in submerged macrophyte beds. This method is easily repeatable, cost effective, relatively quick, and minimises the biases that are introduced by the presence of SCUBA divers (Harvey *et al.*, 2007; Franco *et al.*, 2011). Video based techniques are suitable for clear waters and have proven very useful in assessing the effectiveness of marine protected areas (Harvey *et al.*, 2007). Underwater video analysis is a relatively new technique that has been used in a range of aquatic environments, including marine, estuarine and freshwater habitats (Becker *et al.*, 2010; Pelletier *et al.*, 2011; Ellender *et al.*, 2012; Letessier *et al.*, 2015) and has been successful in assessing fish density, relative abundance, fish assemblages, and behaviour (Cappo *et al.*, 2007; Becker *et al.*, 2010; Lowry *et al.*, 2011; Gladstone *et al.*, 2012; Weyl *et al.*, 2013). Assessing the behaviour of fishes allows for a better understanding as to how different fish species utilize different areas (Becker *et al.*, 2010) and this method was used to assess whether the two different fish families used the two different habitats for different purposes, e.g. feeding or shelter. This non-destructive sampling method is thus a good alternative to other sampling methods when working with fish populations associated with submerged plant beds in clear waters. There are of course drawbacks to this method and this is discussed in detail in the discussion.

1.5 Study objectives and hypotheses

Zostera capensis meadows occupy a large area, approximately 421 hectares of the total estuarine area (1,827 hectares), within the Knysna Estuary (Barnes, 2010b) (see Chapter 2 for details of the study area) but little is known about their importance to associated fish assemblages. With *Z. capensis* meadows being under increased pressure from anthropogenic influences it is important to establish which fish species utilize these ecosystems and what role they play in the ecology of fish assemblages within the Knysna Estuary. The primary objective of this study was to identify the fish species using the *Z. capensis* meadows in this system.

The key questions addressed by this study are;

1. Is a different fish family dominant in *Z. capensis* meadows when compared to the adjacent areas without vegetation?
2. What is the relevant density and behaviour of fishes within *Z. capensis* meadows and adjacent unvegetated areas?

The study site is described in detail in Chapter 2, followed by an explanation of the methods used to collect data pertaining to fish assemblages, including fish species composition and fish behaviour in two different habitats in the Knysna Estuary (Chapter 3). Analysis of the data sets collected in Chapter 3 occurs in Chapter 4 where the differences in behaviour between Sparidae and Mugilidae with respects to the two different habitats, as well as the assemblage composition and abundance of these two fish families during different seasons are examined. Chapter 5 then draws together the results from Chapter 4 and puts the information from this study into context with similar studies conducted elsewhere.

The two main hypotheses addressed by this study are;

1. Mugilidae are likely to be more dominant in the bare, unvegetated areas of the estuary littoral whereas the Sparidae are likely to predominate within the *Z. capensis* bed areas of the estuary littoral.
2. The non-destructive underwater video monitoring method yields similar fish composition data to seine netting sampling of the identical sites.

These hypotheses were selected as it has been documented in other studies (Beckley, 1983; Hanekom and Baird, 1984; Whitfield, 1988; Whitfield *et al*., 1989) that Sparidae generally utilize *Z. capensis* areas whereas Mugilidae feed mostly on bare sediment areas and would therefore be expected to be scarce in *Z. capensis* beds.

Chapter 2 - Study Area

2.1 Physico-chemical attributes of the Knysna Estuary

The Knysna Estuary (34°04′35″S; 23°03′40″E) is located on the southern coast of South Africa and is classified as a warm-temperate estuary (Whitfield, 2000). The estuary has a meandering S-shape lying in a southeast to northwest orientation (Figure 2.1) with a total area of 1827 ha (Grindley, 1985). The permanently open mouth of the estuary is 229 m wide at its widest point, with a 120 m wide and 15 m deep channel between The Heads (two rocky headlands) (Grindley, 1985; Russell *et al.*, 2012). There is a strong current flow through The Heads that can exceed 1.1 m^{-s} s^{-1} during spring tides (Day, 1981). This tidal intrusion extends upstream along the main channel of the estuary for approximately 14 to 18 km (Day, 1981; Russell, 1996; Allanson *et al.*, 2000a). The deep mouth allows for regular replacement of estuarine water, with an outflow of warmer surface waters and a slow entry of colder, more saline bottom waters in the middle and lower reaches (Schumann, 2000; Marker, 2003).

The tidal fluctuations within the estuary are similar to the adjacent marine environment with the tidal range at spring tide at the mouth being 1.8 m (Grindley, 1985) and in the estuary between 0.4 and 1.7 m (Grindley, 1985), which means the natural salinity gradient from head to mouth is maintained (Day, 1981; Allanson *et al.*, 2000a; Largier *et al.*, 2000; Schumann, 2000; Marker, 2003). Average surface water temperatures at the mouth in summer are between 19°C and 23.6°C, except during upwelling events when water temperatures can fall to 10°C (see below), with the average winter temperatures in the lower reaches being 13.9°C to 17.1°C (Day *et al.*, 1951; Day, 1981; Schumann, 2000). Water temperatures in the rest of the estuary range between 11°C in winter and 29°C in summer, with the higher temperatures recorded in the upper reaches (Day, 1981; Russell, 1996). The fresh water temperature flowing in from the Knysna River varies seasonally from 12°C in winter to 27°C in summer which means that the longitudinal temperature gradient that is seen in summer may be reversed in winter months (Largier *et al*., 2000) as the average temperature at the mouth of the estuary is approximately 15.5°C in winter (Day *et al*., 1951; Day, 1981; Schumann, 2000).

Off the Knysna Estuary, the Agulhas Bank forms a wide continental shelf area which has marked seasonal variations in ocean water temperature with strong thermoclines occurring during the summer (Day, 1981; Schumann, 2000). In addition to this, easterly winds (mainly during summer) cause upwelling events and very cold water can enter the estuary, resulting in a decrease in estuarine water temperature of between 10°C and 15°C within one or two days (Day, 1981; Grindley, 1985; Russell, 1996; Allanson *et al*., 2000a; Largier *et al*., 2000; Schuman, 2000). The cold water penetrates far into the estuary (Grindley, 1985) with low temperatures recorded past the Railway Bridge (Largier *et al*., 2000) as far as Belvidere (Allanson *et al*., 2000a). These events are generally short lived (Russell, 1996), with time scales of between one and five days (Schumann, 2000).

The Knysna Estuary has very unique features and is the only estuarine bay type system on the Cape south coast (Day, 1981). The estuary can be divided into three sections: 1) the lower estuary or embayment from the mouth to the railway bridge with strong tidal influences and near oceanic salinities (known as the bay regime), 2) the middle estuary, dominated by warmer waters with a strong longitudinal salinity and temperature gradient from the railway bridge to the White Bridge (N2 road bridge) (known as the lagoon regime) and 3) the upper estuary, influenced by fluvial flow and thus having low-salinity water, from the White Bridge upstream (known as the estuary regime) (Figure 2.1) (Day, 1981; Largier *et al*., 2000).

Approximately 2.5 km from the mouth, Leisure Isle (82 ha, Russell *et al*., 2012) is situated as a consolidated flood-tide sandbank (Largier *et al*., 2000). It is this area between the mouth and Leisure Isle that is known to have the greatest species diversity due to the fauna having both a marine and estuarine component (Day *et al.*, 1951). From here the estuary widens to approximately 3 km (Day, 1981), with water being transported along the Ashmead Channel, which has a depth of approximately 0.85 m to 3.8 m (Prof. B.R. Allanson, pers. comm.) and a width of up to 300 m (Day, 1981), and past Thesen Island (84 ha, Russell *et al.*, 2012) towards the railway bridge. From the railway bridge the water moves along a narrowing and branching channel up to the White Bridge, upstream where tidal currents weaken and the estuary narrows until it meets the Knysna River (Largier *et al.*, 2000). Mid-channel depths within the estuary are 2 to 5 m below the mean low water level and tidal scour holes of 10 to 17 m deep are found within 2 km of the estuary's mouth (Day, 1981; Grindley, 1985; Largier *et al.*, 2000).

In a review by Russell *et al.* (2012) it is reported that the estuary's substratum mostly consists of unconsolidated sandy sediments of aeolian, fluvial and marine origin. Although the Knysna Estuary is a permanently open system, only small amounts of marine sediments enter the estuary and occur mainly near the mouth (Russell *et al.*, 2012). The surface sediments in the lagoon regime area seem to be almost exclusively of aeolian material derived from the Brenton dune on the southern bank of the estuary (Russell *et al.*, 2012). In the estuary regime area (as per Largier *et al.*, 2000 definition), most of the sediments seem to be of fluvial origin due to the angularity and poorly sorted manner of the sand grains, whereas the sediments around Thesen Island and Leisure Isle consist of aeolian transported material and fluvially transported mud (Russell *et al.*, 2012). Day *et al.* (1951) described five main types of substrata in the estuary, including sand and stone at the head of the estuary, sand and rock at Old Drift, mud at Westford Bridge, muddy sand at The Point, Brenton and the Railway Bridge and rock at The Heads (Figure 2.1).

A study of the movement of sediment within the estuary by Marker (2000) has shown that the shore associated with Leisure Isle is prone to erosion during winter under westerly storm conditions when large waves enter the estuary from The Heads. The Point is affected by strong tidal scouring, which is increased by north-westerly storm conditions, while the Brenton shore is affected by strong easterly winds, particularly when accompanied by rain. Recreational activities, such as the use of boats which cause waves, can increase the erosion of these areas in the main channel (Marker, 2000). In the lagoon regime area (as per Largier *et al.*, 2000 definition), sedimentation occurs from runoff from the Knysna and Salt rivers, however erosion can also occur during flooding episodes (Marker, 2000).

Although the long term average rainfall is similar throughout the year (Day *et al.*, 1951; Russell *et al.*, 2012), high rainfall usually occurs during the months of February, March, May, September, November and December (Russell *et al.*, 2012). However, Day *et al.* (1951) recorded high rainfall in the month of July as well. The Knysna Estuary is mainly supplied with fresh water from the perennial 64 km long Knysna River with the average rainfall in its catchment estimated as 928 mm per year (Marker, 2003; Switzer, 2008; Russell *et al.*, 2012). Other sources of freshwater include the smaller Salt River and the Bigai stream (Marker, 2003; Switzer, 2008).

The salinity of the water in the Knysna Estuary shows considerable seasonal variation (Day, 1981; Grindley, 1985) and surface salinities, which increase from the head of the estuary downstream (Allanson *et al.*, 2000a), range from freshwater (0) to marine conditions (36.7) (Grindley, 1985). Salinities at the mouth of the estuary are generally near oceanic, approximately 34 (Largier *et al.*, 2000), decreasing to between 32 and 33 half way up the estuary (Day, 1981) and 30 to 34 at the Railway Bridge (Grindley, 1985; Largier *et al.*, 2000). The salinity falls below 30 in the estuary regime area and has been recorded as zero at the head of the estuary (Grindley, 1985). During reduced freshwater inflow from the river, the salinity in the upper reaches of the estuary can be above 35 (Russell *et al.*, 2012). The temporal variation in salinity is, however, greatest at the head of the estuary at 35.5 and decreases down the estuary, with an average of 31.1 in the estuary regime area, 21.2 in the lagoon regime area and only 13.2 at the mouth (Russell, 1996). The degree of mixing increases down the estuary. There is often a marked vertical salinity gradient in the upper reaches (Day, 1981; Largier *et al.*, 2000), whereas in the lower reaches the water is completely mixed and there is no vertical salinity stratification (Day *et al.*, 1951; Grindley, 1985; Largier *et al.*, 2000).

Russell (1996) recorded mean pH values of 8.3 at the mouth and bay regime, 8.1 in the lagoon regime and 7.5 at the head of the estuary. The highest pH values have been recorded at the mouth of the estuary and in the bay regime (8.8) and lowest in the lagoon regime (6.0) and at the head of the estuary (5.1) (Russell, 1996). Grindley (1985) and Allanson *et al.* (2000a) found a similar range in pH for the estuary, with a minimum of 6.6 and a maximum of 8.5.

The estuarine water is well oxygenated owing to the large tidal range (Day, 1981; Watling and Watling, 1982) with the saturation of the water in the main channel varying between 82 and 97% (Allanson *et al.*, 2000a). The mean concentration of dissolved oxygen decreases longitudinally up the estuary, with a range of 10.2 mg l^{-1} in the bay regime area to 3.1 mg l^{-1} at the head of the estuary (Grindley, 1985; Russell, 1996; Allanson *et al.*, 2000a).

According to Grindley (1985) the nutrient concentrations in the channel north of Thesen Island are high but in the rest of the estuary the nitrate levels decrease from approximately 2.5 µg of $NO_{3\text{-N/l}}$ at The Heads to approximately 0.9 µg per litre in the upper reaches of the estuary during winter. In autumn the reverse is true, with the nitrate concentration increasing from The Heads through to the upper reaches of the estuary. Allanson *et al.* (2000a) found that changes in water column nutrients were more closely related to specific events such as storm water inflows, river flooding and sewage treatment plant effluent, than to seasonal, tidal and regime impacts. NO_xN and soluble reactive phosphate (SRP) concentrations were significantly higher in the Ashmead Channel compared to the main channel due to the inflow of sewage effluent (Allanson *et al.*, 2000a). Allanson *et al.* (2000a), however, showed that there is a seasonal affect, with NH_4N showing an increase in winter and a decrease in NO_xN concentrations. As summer is generally a time of increased sewage effluent inflow, there is an increased loading of NH_4N (Allanson *et al.*, 2000a). Russell *et al.* (2012) reported that certain of Knysna's drains are responsible for the input of nickel, zinc, mercury, cobalt and copper but that their ecological impact on the estuary was insignificant. Watling and Watling (1982) examined metal concentrations in water, sediment and mollusc samples from the estuary (1975 to 1978) and found that the estuary was relatively unpolluted. A spring storm in 2000 allowed Switzer (2008) to track urea concentrations both pre- and post-storm to demonstrate the initial and long-term impacts of urea loading in the estuary. It was found that storms have a significant influence on nutrient availability as the urea concentrations in the rivers that supply the estuary with fresh water increase drastically owing to informal housing settlements and farming practices. The increase in nutrient availability could be related to the large dinoflagellate blooms that are often observed in the estuary in the late summer (Switzer, 2008).

The phytoplankton chlorophyll-*a* concentrations in the estuary are generally low owing to the water column being predominantly oligotrophic (Allanson *et al.*, 2000a; Russell *et al.*, 2012), but there is a seasonal difference, with summer having higher mean concentrations (3.01 μg^{-l}) than winter (1.37 μg^{-l}) (Allanson *et al.*, 2000a). Water clarity decreases from the mouth towards the head waters (Russell, 1996) with a seasonal variation being apparent (Allanson *et al.*, 2000a).

The bay regime area has a high Secchi disc transparency (2.3 ±1.05 m) with a decreased transparency in the lagoon (1.5 m) and estuary (1.7 m) regime areas (Day, 1981; Grindley, 1985; Allanson *et al.*, 2000a). The lagoon and estuary regimes experience lower transparency during the summer months owing to increased river run off during the rainy season (Allanson *et al.*, 2000a) and floods can occur during the early summer wet season (Switzer, 2008).

2.2 Ecological research in the Knysna Estuary

The Knysna Estuary has been described as having the richest biodiversity of all estuaries along the South African coast owing to a wide range of habitats being colonised by a variety of plant and animal taxa (Day, 1981; Grindley, 1985; Allanson *et al.*, 2000a; Maree, 2000; Barnes, 2010b;

Adams, 2016). The estuary is ranked number one in the top 50 South African estuaries in terms of conservation importance (Turpie *et al.*, 2002; Russell *et al.*, 2012; Adams, 2016) and conserves a total of 501 species (Turpie *et al.*, 2002). The rich biodiversity of the estuary accounts for 42.7% of South Africa's total estuarine biodiversity (Turpie, 2000; Barnes, 2010b).

The earliest detailed ecological data for the Knysna Estuary were from Day *et al.* (1951) and Korringa (1956) who focussed on water column characteristics and invertebrate species richness and zonation. Allanson *et al.* (2000b) compared benthic macrofaunal richness and diversity between 1997 and data published by Day *et al.* (1951), and found that in the 50 years between surveys there had been no change in species richness or diversity within the soft intertidal sediments. However, there was a significant increase in species diversity in sediments sampled in the *Zostera* zone, possibly caused by an increase in suspensoids in the water column from poor land use management practices which made the sediments more suitable to species of resident taxa as well as new taxa through altering the quality of the sediments (Allanson *et al.*, 2000b). Hodgson *et al.* (2000a) looked at the intertidal population structure and standing stock of the mud prawn *Upogebia africana* in the Knysna Estuary and recorded that mud prawn biomass and density was generally higher in *Spartina* and lower *Zostera* zones with wide distribution from north of Leisure Isle to the Red Bridge. Hodgson *et al.* (2000b) looked at the exploitation of mud prawns as bait in the estuary during 1995 and 1996 and showed that approximately 0.9% of the entire estuary stock of *U. africana* is removed annually. The estuary supports more than 340 benthic invertebrate species (Day *et al.*, 1951; Barnes, 2010b) although species diversity decreases progressively from the mouth to the head of the estuary (Grindley, 1985; Russell *et al.*, 2012).

Grindley (1985) documented 60 fish species to be common in the estuary although there is reputed to be 200 fish species (Bulpin, 1978). *Rhabdosargus holubi, Lithognathus lithognathus, Argyrosomus japonicus, Lichia amia* and several Mugilidae and other Sparidae species were common (Grindley, 1985). Le Quesne (2000) recorded 26 fish species in the intertidal estuarine marshes of the Knysna

Estuary, with the Mugilidae being the dominant family, making up 45% of the catch and the Sparidae the second most dominant at 36%.

Grindley (1985) also reviewed information on the avifauna of the Knysna Estuary, documenting 74 waterbird species associated with the estuary. In his review, Grindley (1985) also reports on the count done by Underhill *et al.* in 1980 where a total of 3889 birds were counted, of which 75 were non-Palearctic waders, 2799 Palearctic waders and 1015 non-waders. Martin *et al.* (2000) conducted a comprehensive study on the waterbirds of the Knysna Estuary and found that waterbird numbers were highest during summer with a median of 5343 compared to a median of 2336 in winter. In summer 76% of the avifauna comprised Palearctic migrant terns and waders, of which 95% migrate in winter and thus winter waterbird numbers are about 44% of summer numbers (Martin *et al.*, 2000). Fifty-four waterbird species were recorded in the winter and 57 species in summer with a total of 67 species recorded on the estuary comprising of 17 Palearctic migrant species and 50 resident species (Martin *et al.*, 2000). Resident waterbird numbers increased by 63% during winter, from a summer median of 1292 to a winter median of 2104 (Martin *et al.*, 2000). Summer avifauna consisted predominantly of invertebrate-feeding migrant waders such as Curlew Sandpipers, Grey Plover, Greenshank and Whimbrel, while winter avifauna mainly consisted of piscivorous non-migratory species such as Kelp Gull, Cape Cormorant, Reed Cormorant, Little Egret and Sacred Ibis (Martin *et al.*, 2000).

Approximately 1000 ha of the estuary comprises wetlands, occurring from Leisure Isle up to 12.5 km from the mouth to the Westford Bridge (Le Quesne, 2000; Maree, 2000; Mucina *et al.*, 2006; Russell e*t al.*, 2012). The marshes in the lower and middle reaches of the estuary occupy approximately 750 ha, which is about 44% of the water surface area of the tidal estuary, and play an important role in nitrogen metabolism of the entire ecosystem (Allanson *et al.*, 2000a; Maree, 2000). This habitat occurs up to 8.5 km from the mouth on the west bank to Belvidere and 7 km from the mouth on the east bank to the Point (Figure 2.1) (Maree, 2000). At George Rex Drive an intertidal saltmarsh area of 90 ha is found, at Thesen Island the saltmarsh occupies 80 ha and extensive intertidal marsh areas are found on the western shore of the estuary at Brenton (Maree, 2000). The intertidal marshes consist of communities of *Limonium linifolium, Triglochin* spp., *Sarcocornia perennis* and *Spartina maritima* (Grindley, 1985; Maree, 2000; Russell *et al.*, 2012). The supratidal marsh consists of *Plantago crassifolia* and *Chenolea diffusa* and cover 60 ha on Thesen Island, west of George Rex Drive, The Point and Belvidere, with the largest area of 18 ha found at Brenton (Figure 2.1) (Maree, 2000; Russell *et al.*, 2012).

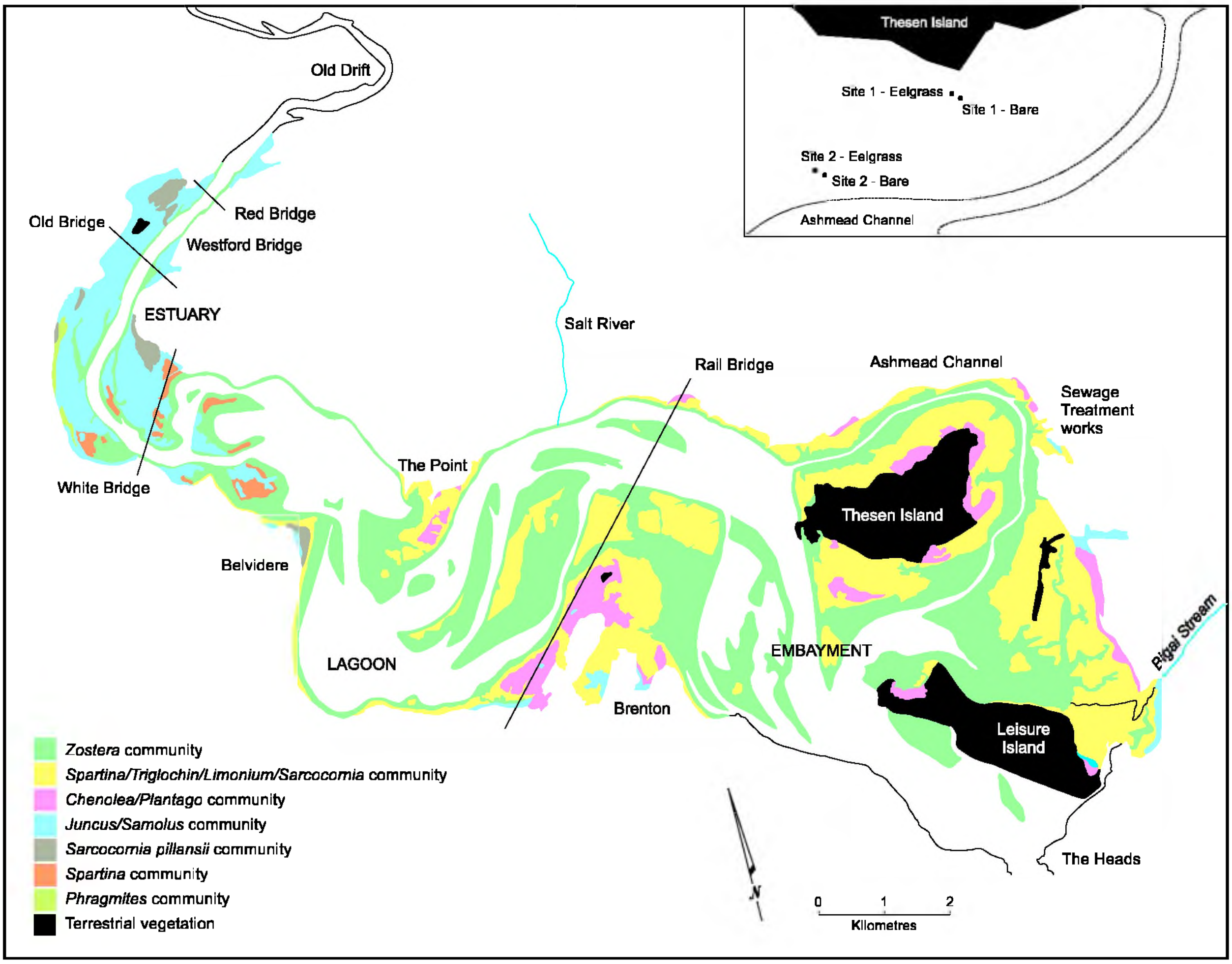

Figure 2.1: Map showing the distribution of the main plant community types in the Knysna Estuary as well as the three different regimes and the main areas in and around the estuary (summarized from Allanson *et al.*, 2000a; Largier *et al.*, 2000; Maree, 2000). The inset shows the location of the camera recording sites and seine netting site between Thesen Island and the Ashmead Channel.

In the upper reaches of the estuary the marshes are dominated by *Juncus kraussii* (Grindley, 1985; Le Quesne, 2000), occupying approximately 150 ha between Crab's Creek and the Westford Bridge (Maree, 2000; Mucina *et al*., 2006). From the Westford Bridge upstream, the submerged macrophyte *Ruppia maritima* is common, replacing *Z. capensis* and reaches its highest density at the Old Drift (Grindley, 1985; Russell *et al*., 2012). These areas are important to the ecology of the estuary as they provide habitat for many organisms including fish, a wide range of invertebrates and birds, as well as playing an important role as a nutrient sink during the recycling of nitrates and phosphates (Maree, 2000).

Throughout the estuary there is a zonation of plants as well as animals (Day *et al*., 1951). Different plants and animals are restricted to particular tidal levels on the banks which can be grouped into three zones, namely the *Juncus-Salicornia* zone, the *Puccinella* (now known as *Spartina*) or bare zone and the *Zostera-Upogebia* zone (Day *et al*., 1951). Each of these zones are characterized by particular animals (Figure 2.2).

Knysna Estuary has the largest area of *Zostera capensis* Setchell meadows in South Africa (49% of the national total) which is dominated by *Z. capensis* (Grindley, 1985; Russell *et al*., 2012) and cover 66 ha of the intertidal zone and 355 ha of the subtidal zone in the estuary (Mucina *et al*., 2006; Barnes, 2010b; Adams, 2016). There are extensive *Z. capensis* meadows around the Ashmead channel, where they are up to 200 m wide (Day, 1981; Allanson *et al*., 2000a; Maree, 2000), Thesen Island and north of the railway bridge (Figure 2.1) (Maree, 2000). Unfortunately the *Z. capensis* beds are heavily impacted by bait collection in some areas (Hodgson *et al*., 2000b; Maree, 2000; Barnes, 2010b; Russell *et al*., 2012) because certain bait collectors use garden forks and spades to dig trenches 8 to 20 cm deep, 2 to 3 m long and up to 1 m wide (Hodgson *et al*., 2000b; Russell *et al*., 2012). These areas take more than three years to recover from this kind of disturbance and this results in changes in the infaunal community composition (Allanson *et al*., 2000b; Hodgson *et al*., 2000b; Maree, 2000).

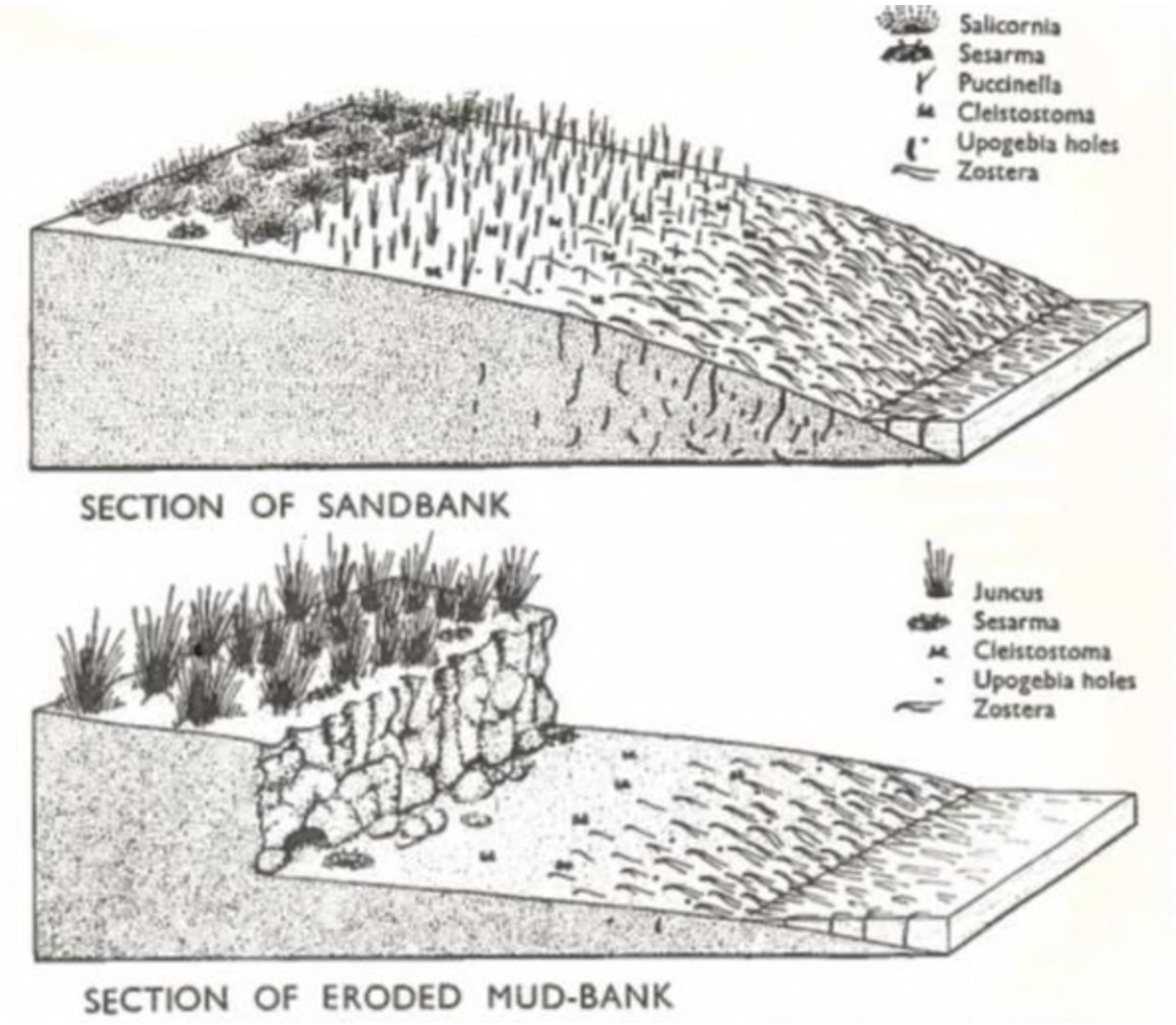

Figure 2.2: Two types of bank found in the Knysna Estuary with the main types of animals and plants associated with them (diagrams reproduced from Day *et al.* 1951) (Permission granted to use diagrams by Taylor and Francis Group).

The *Z. capensis* meadows in Knysna Estuary and the fauna that it supports are of very high conservation importance (Barnes and Ellwood, 2012). Surveys have shown that many macrofaunal species are associated with the *Z. capensis* beds (Barnes, 2010a), living on the *Z. capensis* leaves or in or on the sediment within these beds (Barnes, 2010a). The macrobenthos plays a very important role in the food webs of *Z. capensis* meadows and are important to the significant *Z. capensis* dependent fisheries within the Knysna Estuary and further afield (Barnes, 2010b).

The most prominent invertebrate taxa associated with the Knysna *Z. capensis* beds include isopods, amphipods, molluscs, polychaetes, oligochaetes and brachyuran crustaceans (Barnes, 2010a; Barnes, 2010b). A total of 122 macrofaunal species have been recorded in the *Z. capensis* beds of the Knysna Estuary with an estimated total species richness of 151 (Barnes, 2013). Of these the most abundant species include *Ceratonereis erythraeenis, Perinereis nuntia, Aquilaspio sexoculata, Turritella capensis, Hydrobia knysnaensis, Rissoa capensis, Cleistostoma edwardii and Halmyrapseudes digitalis* (Barnes, 2010b). Over the past 54 years it has been estimated that the area of *Z. capensis* in the Knysna Estuary has been reduced by 26% (Barnes, 2010b).

2.3 Study sites

The sites for this study were situated in the lower reach of the Knysna Estuary between Thesen Island and Leisure Isle (see inset in Figure 2.1). One site was in a small channel close to Thesen Island and the other closer to the Ashmead Channel. Each site had a *Zostera capensis* bed and a bare, unvegetated area. At Site 1 the *Z. capensis* bed and bare unvegetated area was 21.7 meters apart while at Site 2 the *Z. capensis* bed and bare unvegetated area was 24.5 meters apart.

Site 1: *Zostera capensis*- 34° 03′208″ S 23° 03′301″ E; Bare- 34° 03′215″ S 23° 03′318″ E

Site 2: *Zostera capensis*- 34° 03′235″ S 23°03′373″ E; Bare- 34°03′239″ S 23° 03′369″ E.

These sites were chosen as water clarity in this part of the estuary was good enough for the use of underwater video monitoring, and the security measures on Thesen Island ensured that the cameras would not be subjected to human interference. Another positive attribute of these sites was that there was no need for the use of a boat to get to the sites which meant less disturbance to fish in the area and seine netting was possible because the intertidal banks enabled beaching of the seine net.

At the beginning of the study in January 2014, the two *Z. capensis* sites were completely covered with *Z. capensis* (pers. obs.). Site 1 had *Z. capensis* with long, wide blades (Figure 2.3) while Site 2 had *Z. capensis* with shorter, narrower blades and there were signs of bait digging (pers. obs.) (Figure 2.4). However, in the summer of 2015 an *Ulva* bloom occurred that extended into most of the study area, covering some intertidal areas completely (B.R. Allanson, pers. comm.). The *Ulva* bloom consisted of two species, *Ulva lactuca* and *U. intestinalis* (J. Adams, pers. comm.). This *Ulva* bloom lasted through most of 2015 and impacted the two sampling sites, so that by the end of sampling in August 2015 the two *Z. capensis* sites had changed completely in terms of vegetation composition. Site 1 had very little *Z. capensis* left, with large areas completely covered with *Ulva* or left bare as the *Z. capensis* had undergone senescence and not recovered (Figure 2.5). There were also signs of bait digging at Site 1 which could account for some of the bare areas (pers. obs.) (Figure 2.5). Site 2 had a mixture of *Z. capensis* and *Ulva* as well as larger areas of bare sand (Figure 2.6), the latter resulting from increased bait digging in this area (pers. obs.). It should be noted that *Z. capensis* is a seasonal plant, being more dense and extensive in summer than in winter months (Kaletja and Hockey, 1991). The change in vegetation composition (Figures 2.3 - 2.6), could in part be due to seasonal effects but data collected in August 2014 and August 2015 suggest that *Ulva* also played a role in *Z. capensis* dynamics at the study sites.

Macroalgal blooms such as *Ulva* have been known to damage seagrass beds and can have a negative effect on the resident organisms in the area as it results in low dissolved oxygen levels at night and a significant reduction in light penetration under and within these algal mats (Sogard and Able, 1991; Watson *et al.*, 2015). *Ulva* grows on gravel, rocks and shells and detaches easily to proliferate while it floats (Day, 1981). When it is cast ashore by wind and waves it becomes a public nuisance (Day, 1981), with the decaying mass being associated with ammonium and sulphide accumulation, low redox potentials and anoxia which is detrimental to infauna and seagrass associated biota (Watson *et al.*, 2015).

Figure 2.3: Site 1 *Zostera capensis* beds in January 2014.

Figure 2.4: Site 2 *Zostera capensis* beds in January 2014.

Figure 2.5: Site 1 *Zostera capensis* beds in August 2015.

Figure 2.6: Site 2 *Zostera capensis* beds in August 2015.

Chapter 3 - Materials and Methods

3.1 Underwater visual monitoring

3.1.1 Pilot study

A method for the deployment of cameras was first tested at the beginning of January 2014. At that time two GoPro® HD Hero® 3+ high-definition cameras were available. Deployment of cameras at low tide was not feasible as water turbidity was found to be too high for a clear picture during recordings. Therefore, the cameras were deployed over high tide. At first, each camera was attached approximately one meter above the substratum with a GoPro® clamp to a 4 cm diameter white PVC pole that was pushed vertically into the substratum. This method did not work as the water currents during the ebb and flow were too strong and pushed the poles over. Therefore, metal stands were constructed to support the PVC poles in the substratum. These stands consisted of a cylindrical middle piece (5.5 cm diameter) to house the PVC pole, surrounded by four equally spaced metal wings each with a length of 15 cm and depth of 20 cm (Figure 3.1). This made the diameter of the whole stand 35.5 cm. The metal stands were painted with Duram NS4 Anti Rust Coating Red Oxide to prevent them from rusting. Each stand with a PVC pole was pushed into the substratum and they were deployed for the first time on the 30th of January 2014. With these stands the PVC poles remained vertical and it was easy to insert and extract them from the substratum.

The stands, with the poles and attached cameras, were deployed multiple times between January and July 2014 but no fish were recorded. It was suggested (Professor A.K. Whitfield, pers. comm.) that the white PVC poles were deterring fish and the poles were therefore painted dark green with Duram Rainkote Fibre-Tech paint (matt finish). In addition, the underwater housing of each camera was covered with fine black netting with an opening left for the lens.

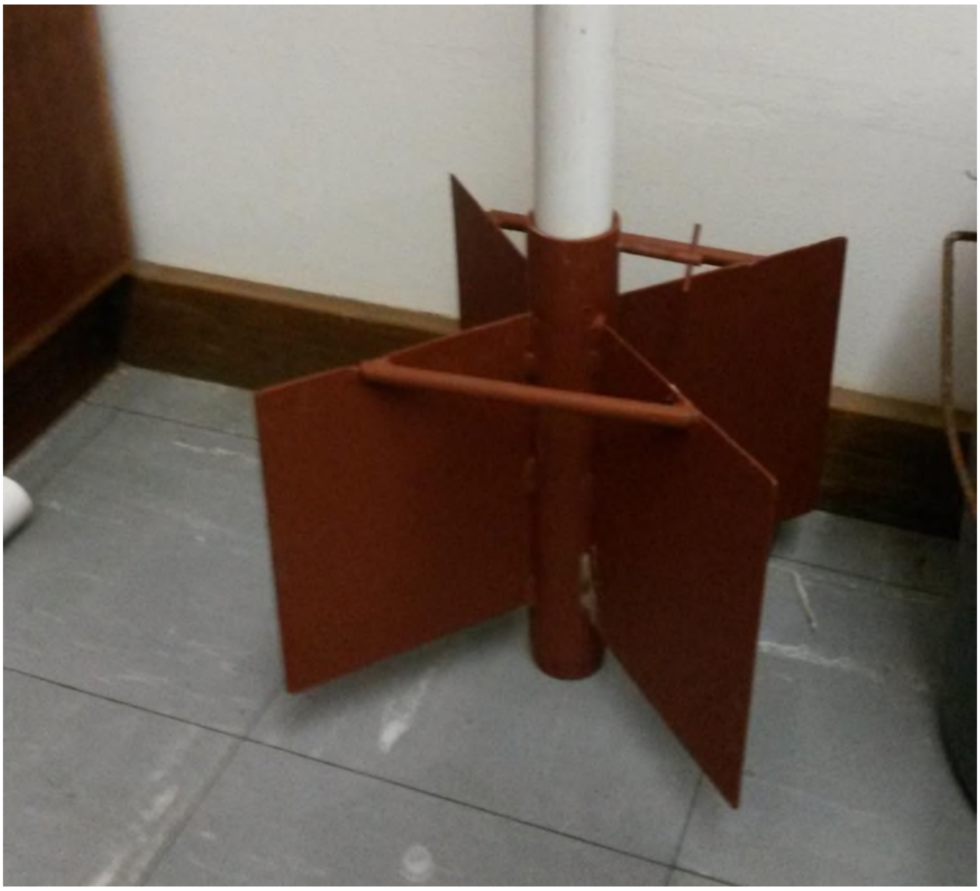

Figure 3.1: The 35.5 cm metal stand painted with Duram NS4 Anti Rust Coating Red Oxide along with the white PVC pole.

The new camouflaged poles and cameras were deployed for the first time on the 4th August 2014 and fish were seen in video footage for the first time. A second site was added to the study area on the 16th August 2014 with the two cameras alternating on different days between sites (one camera in a *Z. capensis* bed and one in a bare unvegetated area). Two additional cameras (GoPro® HD Hero® 3) were added on the 18th October 2014, so that the two sites could be recorded at the same time. The pilot study for the testing of equipment covered the period 15th January 2014 until the 4th August 2014, with project data collection from video footage at both sites commencing on the 16th August 2014 and ending on the 11th August 2015. During each deployment the weather conditions were recorded and water temperature data was sourced from SANParks and The Knysna Basin Project.

3.1.2 Camera settings

Camera settings were standardised: field of view was set on wide (170°), resolution (Full HD) = 270 pixels (1280 x 720; 16:9), frames per second = 60. Each camera had an extra battery pack that enabled the cameras to record for up to 2.5 hours at a time and the videos were saved on 64 GB SanDisk Ultra micro SD flash memory cards. The cameras were set at a 35° angle towards the substratum which was optimal for viewing fish on the substratum and within the water column.

3.1.3 Sampling duration and timing

As the cameras were able to record for up to 2.5 hours at a time, it was decided that recording would take place an hour before and an hour after high tide as water transparency was found to be best at that time. To ensure that there was sufficient light to view fish in the camera footage, deployment took place during high tides in the day. Russel (1996) recorded the turbidity of the Knysna Estuary and found a range of 1.3 to 126 NTU. For recording purposes it was estimated that an NTU of below 50 would still produce a clear enough picture to identify fish species on the video recording. Although no turbidity measurements were taken during sampling, if a video recording was not clear enough to identify fish to species level, that recording was discarded. On cloudy days with rain, or if strong winds prevailed, the water turbidity was generally too high for a clear picture; thus sampling was often restricted by weather conditions to sunny days with light winds. As the sampling could only take place when weather conditions were suitable, recording could not be based on tidal cycles.

Recordings were undertaken in four seasons. Spring sampling took place during October and November 2014 between 06:00 and 17:00, summer sampling during January and February 2015 between 06:20 and 13:00, autumn sampling during April and May 2015 between 07:00 and 13:50, and winter sampling in July and August 2015 between 08:00 and 15:30. There were a total of 18 sampling trips which produced 40 days of recordings (33 days of recordings with all four cameras). This resulted in 8 days of recordings for spring, 7 days for summer, 9 days for autumn and 9 days for winter. As mentioned above, sampling was restricted to days with suitable weather conditions as this influenced sampling success and thus there was a slight difference in effort among seasons.

During summer sampling an *Ulva* bloom occurred in the estuary, thus changing the vegetation composition of the *Z. capensis* sampling sites from predominantly *Z. capensis* to a combination of fragmented *Z. capensis*, *Ulva* spp. and bare sediment, while changing the predominantly bare substratum sampling sites to a combination of *Ulva* spp. and bare sediment. It was therefore decided to compare the data from August 2014 to the data from August 2015 to determine whether the presence of *Ulva* had an impact on fish diversity and abundance at the study sites.

3.1.4 Camera footage analysis

Each 2 to 2.5 hour video was subdivided into 10 minute intervals, thus producing 14 to 15 segments per recording. The first 10 minutes of the video was excluded from the analysis as it was regarded as an acclimation period for conditions in the area to return to normal after deployment (Weyl *et al.*, 2013). In addition the last 10 minutes of recording was excluded to account for any disturbance caused when retrieving the cameras. This resulted in 12 to 13 segments that could be used for species identification but for standardization the first 12 segments, after the exclusion of the first 10 minute section, were used and any segments after these 12 segments were discarded. For each 10 minute segment the MaxN index was recorded for Sparidae and Mugilidae. Identification of these fishes was conducted at family level only as identifying mugilids to species is very difficult using video footage (Becker *et al.*, 2012). Fishes from other families were also recorded but, as this study was focused on only the Sparidae and Mugilidae, these other fish were not included in the analyses. However, a complete list of species observed during recordings is given in Appendix 2.

The MaxN index was used to calculate the relative abundance of species which is the maximum number of individuals for each species or family present in the field of view at the same time (Becker *et al.*, 2010; Becker *et al.*, 2011; Ellender *et al.*, 2012). This is a conservative estimate of relative abundance but it eliminates the chances of counting the same fish twice (Becker *et al.*, 2010; Becker *et al.*, 2011; Ellender *et al.*, 2012). The 12 MaxN index counts thus allowed for a mean MaxN to be calculated for each family for each two hour recording, and is known as mMaxN (Becker *et al.*, 2011).

3.2 Fish behaviour

Data on fish behaviour was recorded following the method of Becker *et al.* (2010). Using this method, the behaviour of each fish observed in each 10 minute video segment was categorised into four broad types as described by Becker *et al.*, (2010). These were, (1) rapid swimming (rapidly passing through the field of view), (2) slow meandering (meandering through the field of view without stopping), (3) stop start (swimming into view, stopping and then moving on), and (4) feeding behaviour (clearly feeding off the benthos or in the water column). According to the above method, when two of the behaviour categories are displayed at the same time by two different fish, the behaviour displayed for the majority of the time by the fish was recorded for analytical purposes.

3.3 Seine netting

Seine netting only took place at the site closest to Thesen Island (Site 1) as at this site it was easier to deploy and beach the seine net. Using the seine net at Site 1 also allowed enough time for the cameras to record for at least two hours before the start of seine netting. There was insufficient time to also sample Site 2 because the tide went out too rapidly and did not leave enough time to conduct netting at both sites. The area swept by the seine net at Site 1 had intermediate vegetative cover, with both *Z. capensis* and bare unvegetated areas present; thus seine netting could not distinguish between fish caught in *Z. capensis* beds and fish caught over bare unvegetated areas. However, the primary aim of the seine netting was to document whether the cameras recorded the same fish species as those captured in the seine net. A complete list of species caught during seine netting is given in Appendix 2.

A 50 m seine net (2 m deep, 15 mm bar mesh) was used to sample the area in the immediate vicinity of the cameras at Site 1 at the start of the ebb tide. Each sampling effort consisted of three seine replicates conducted immediately after each other. All fish caught were identified to species level and then released. Sampling took place over a total of eight days, four days coinciding with a marine upwelling event in the estuary, and four days conducted when warm water temperatures prevailed. The upwelling sampling took place on four mornings in November 2014 when water temperatures were between 11.3°C and 16.6°C. Non-upwelling sampling took place on four mornings between January 2015 and February 2015 when water temperatures were between 21.8°C and 23.4°C.

3.4 Historical data

Unpublished netting data on the fishes associated with the Knysna Estuary was made available by Professor A.K Whitfield (South African Institute for Aquatic Biodiversity). This monthly sampling data was collected by the late Dr H.M. Kok (Port Elizabeth Museum) during 1978, 1979 and 1980 and comprised of 723 sampling stations along the entire length of the estuary, separated into five zones (Figure 3.2). All sampling stations were classified according to the substratum type and consisted of various submerged plant taxa, as well as muddy, sandy and rocky areas. The five zones within the estuary were: Zone 1 – from the Head of the estuary to just above the Old Red Bridge, Zone 2 – from just above the Old Red Bridge to just below the White Bridge, Zone 3 – from just below the White Bridge to the Railway Bridge, Zone 4 – From the Railway Bridge to Leisure Isle, Zone 5 – From Leisure Isle to the mouth of the estuary at The Heads (Figure 3.2).

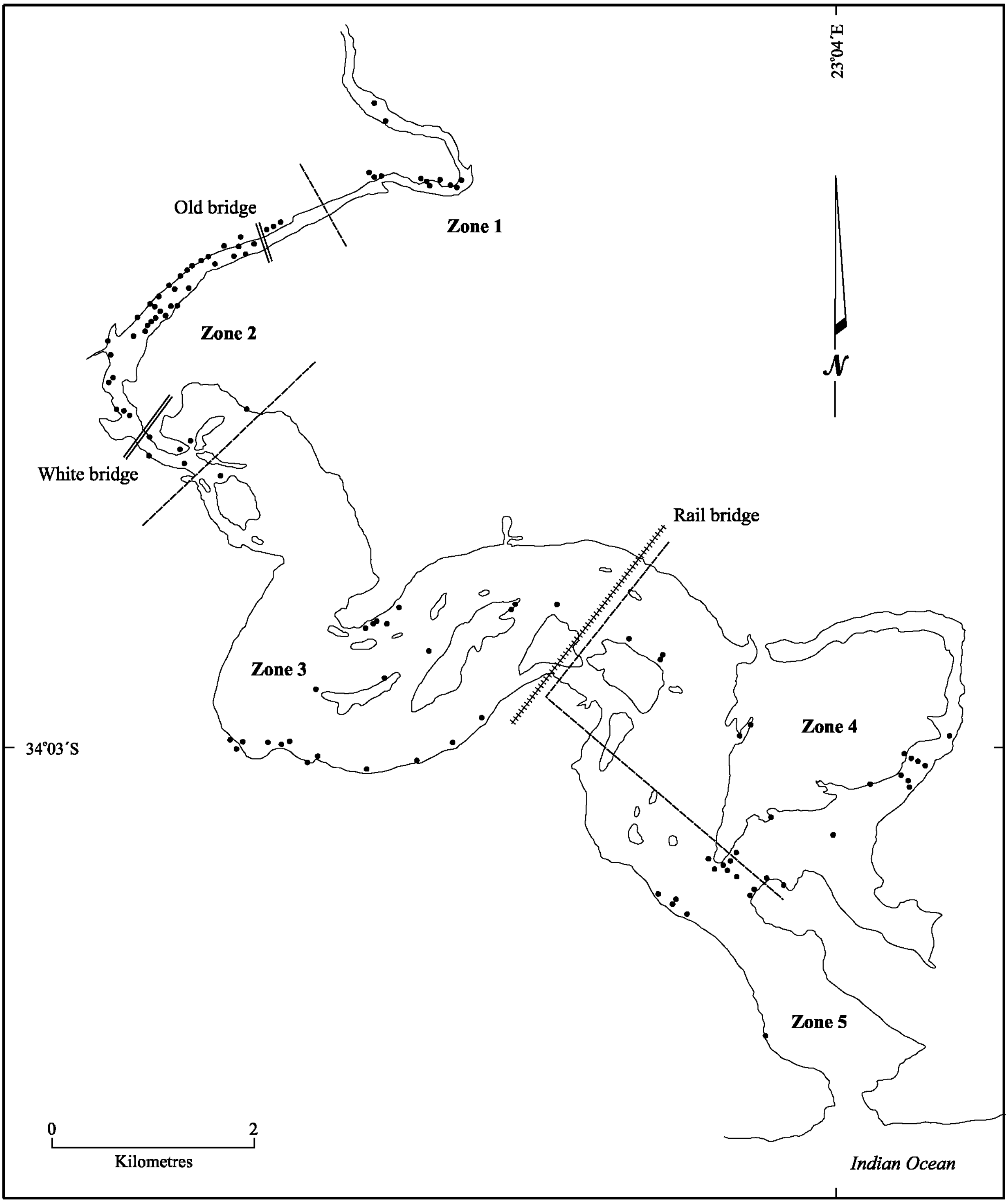

Figure 3.2: Map of the Knysna Estuary and fish sampling stations (used by this study) showing the five zones identified by Dr H.M. Kok. Sampling sites used for the current study indicated by black dots (•).

Fish sampling included multiple net types being used, depending on the area being surveyed. These included cast nets, fry scoop nets, fry seine net (3 m x 1 m x 2 mm bar), drag purse seine net (30 m x 1.6 m x 6 mm bar in the bag and 12 mm bar in wings), monofilament seine net without a bag, and a gill-net. All fish captured during sampling were identified to species level. In addition, at each sampling site the date, zone, habitat type, weather conditions and water temperature were recorded along with co-ordinates that allowed for the specific sampling site to be pinpointed on a map of the estuary (Figure 3.2).

For the purposes of this study the fish data was only analysed for samples that were collected in *Z. capensis* (separated into dense and sparse *Zostera*) and bare unvegetated areas using the drag purse seine net. This produced a total of 170 samples between 1978 and 1980, consisting of fish species and their numbers recorded from 34 dense *Z. capensis,* 103 sparse *Z. capensis* and 33 unvegetated samples. Of the 170 samples, 70 were documented in 1978, 49 in 1979 and 51 in 1980. The contrasting habitats within each of the five zones were not equally balanced for *Z. capensis* and unvegetated sites (see Table 3.1 for details). All the fish recorded in these samples were documented in an Excel spreadsheet for easier processing and the data were separated according to the five different zones. Thereafter a subset of species was created for only the Mugilidae and Sparidae for statistical analysis.

Table 3.1: The number of samples for *Zostera capensis* and bare unvegetated areas in all five zones

Zone	Total samples	Dense *Zostera capensis* samples	Sparse *Zostera capensis* samples	Bare substratum samples
1	16	2	5	9
2	49	5	26	18
3	37	10	24	3
4	33	10	22	1
5	35	7	26	2

3.5 Statistical analyses

3.5.1 Underwater visual monitoring

Generalized Estimating Equations (GEE) were used to run a General Linear Model (GLM) in the statistical program IBM SPSS v 23. The GLM was run with mMaxN as the dependent variable. The data were edited to account for the Hessian matrix and a Poisson link was used to assess whether there were any differences between habitats, families and seasons as well as to see whether there were any significant interactions between these factors. Graphs were produced using SigmaPlot v 12.5. Owing to the nature of the data, there was insufficient variability in the number of individuals for a seasonal analysis to be conducted (e.g. in some seasons there were no mugilids or sparids recorded). Therefore, the data were represented by graphs created in the software program Microsoft Office Excel 2013.

3.5.2 Ulva versus no Ulva

Generalized Estimating Equations (GEE) were used to run a General Linear Model (GLM) in the statistical program IBM SPSS v 23. The GLM was run with mMaxN as the dependent variable. The data were edited to account for the Hessian matrix and a Negative binomial link was more applicable to assess whether there were any differences between habitats, families and seasons as well as to see whether there were any significant interactions between these factors. Graphs were produced using SigmaPlot v 12.5.

3.5.3 Fish behaviour

Generalized Estimating Equations (GEE) were run separately on three (feeding, rapid swimming and slow meandering) of the four different behaviours as there was no counts for the stop-start behaviour. The GLM was run in the statistical program IBM SPSS v 23 with MaxN as the dependent variable. A Negative binomial link was more applicable to assess whether there were any differences between habitats, families and seasons as well as to see whether there were any significant interactions between these factors. Graphs were produced using SigmaPlot v 12.5.

3.5.4 Underwater visual monitoring versus seine netting

As the number of fish observed on video (MaxN) and the number of fish caught (mean number of both Mugilidae and Sparidae caught) are not directly comparable, the proportion of fish species observed in video footage and the proportion of fish species caught by the seine net were calculated. A non-parametric Spearman's rank correlation test was run on the proportional data in SPSS v 23 to establish whether there was any significant correlation between the two sampling methods of underwater visual monitoring and seine netting. A scatterplot graph was produced using Microsoft Office Excel 2013 to represent the correlation. Bar graphs were also produced using Excel 2013 to illustrate the patterns of fish species occurrence using the two different sampling methods under differing water temperature conditions.

A Cook's Distance test was used to check for influence points that might change the outcome of the Spearman's rank correlation as on one day of sampling both the video footage and seine net captured a large number of sparids. The influence points detected by the test were then removed and the Spearman's rank correlation was done on the new data set to see what difference it made to the correlation and R^2 value.

3.5.5 Historical

As these data included the species names of all fish sampled, species level comparisons were undertaken using different statistical tests. The data were coded for habitat (sparse and dense *Z. capensis* and bare substratum), locality (bay, lagoon and estuary) and season (spring, summer, autumn and winter). The localities explain the different physico-chemical factors in three sections of the estuary and is as follows: 1) the lower estuary or embayment from the mouth to the railway bridge with strong tidal influences and near oceanic salinities (known as the bay regime), 2) the middle estuary, dominated by warmer waters with a strong longitudinal salinity and temperature gradient from the railway bridge to the White Bridge (known as the lagoon regime) and 3) the upper estuary, influenced by fluvial flow and thus having low-salinity water, from the White Bridge upstream (known as the estuary regime) (Figure 2.1) (Day, 1981; Largier *et al*., 2000).

The coded data explains the fish numbers in terms of in which habit, locality and season they were recorded. The data were entered into the statistical program PRIMER 6+ and fourth root transformations were applied as it was more applicable than a square root transformation to all fish abundance data as a pre-treatment to reduce the influence of dominant species. A resemblance matrix was created using the Bray Curtis similarity analysis package. Non-metric Multi-Dimensional Scaling (MDS) plots were produced for the factors (habitat, locality and season) respectively using the Kruskal stress formula. An Analysis of Similarities (ANOSIM) was run on all three factors using the Two-Way Crossed Analysis to test for the significance levels and sources of difference between the different factors. Similarity Percentages (SIMPER) analyses were also run on all the factors using the Two-Way Analysis to identify which species contributed most to intra-group similarity and inter-group dissimilarity.

Chapter 4 - Results

4.1. Underwater visual monitoring

Generalized Estimating Equations (GEE) used to run a General Linear Model (GLM) on the pooled underwater visual monitoring data (MaxN) showed that there was no significant difference between habitat type (*Zostera capensis* and bare) and family (Mugilidae and Sparidae) interactions ($\chi^2_{1.3301}$=3.447, p=0.063). There was, however, a significant difference between fish family mean number (MaxN) at *Z. capensis* and bare substratum habitats ($\chi^2_{1.3301}$=12.566, p<0.001), with a significantly higher mean MaxN of Mugilidae (0.28 ± 0.008 SE) observed at bare substratum sites compared to the mean MaxN of Sparidae (0.11 ± 0.011 SE) at the same sites. There was no significant difference in MaxN between the two families observed at *Z. capensis* sites.

Statistical analyses according to habitat, family, and the interaction between habitat and family, could be reported on but not on a seasonal basis owing to insufficient samples in some seasons. However, there was a higher average number of Sparidae observed in summer and spring than in autumn and winter at the *Z. capensis* sites (Figure 4.1 A). The number of Mugilidae were consistently low at these sites during all four seasons (Figure 4.1 A). At the bare substratum sites the Sparidae had higher average numbers in autumn than in summer and winter, with the lowest numbers in spring (Figure 4.1 B). The Mugilidae had slightly higher average numbers in spring and winter, and lower averages in summer and autumn at the bare substratum sites (Figure 4.1 B).

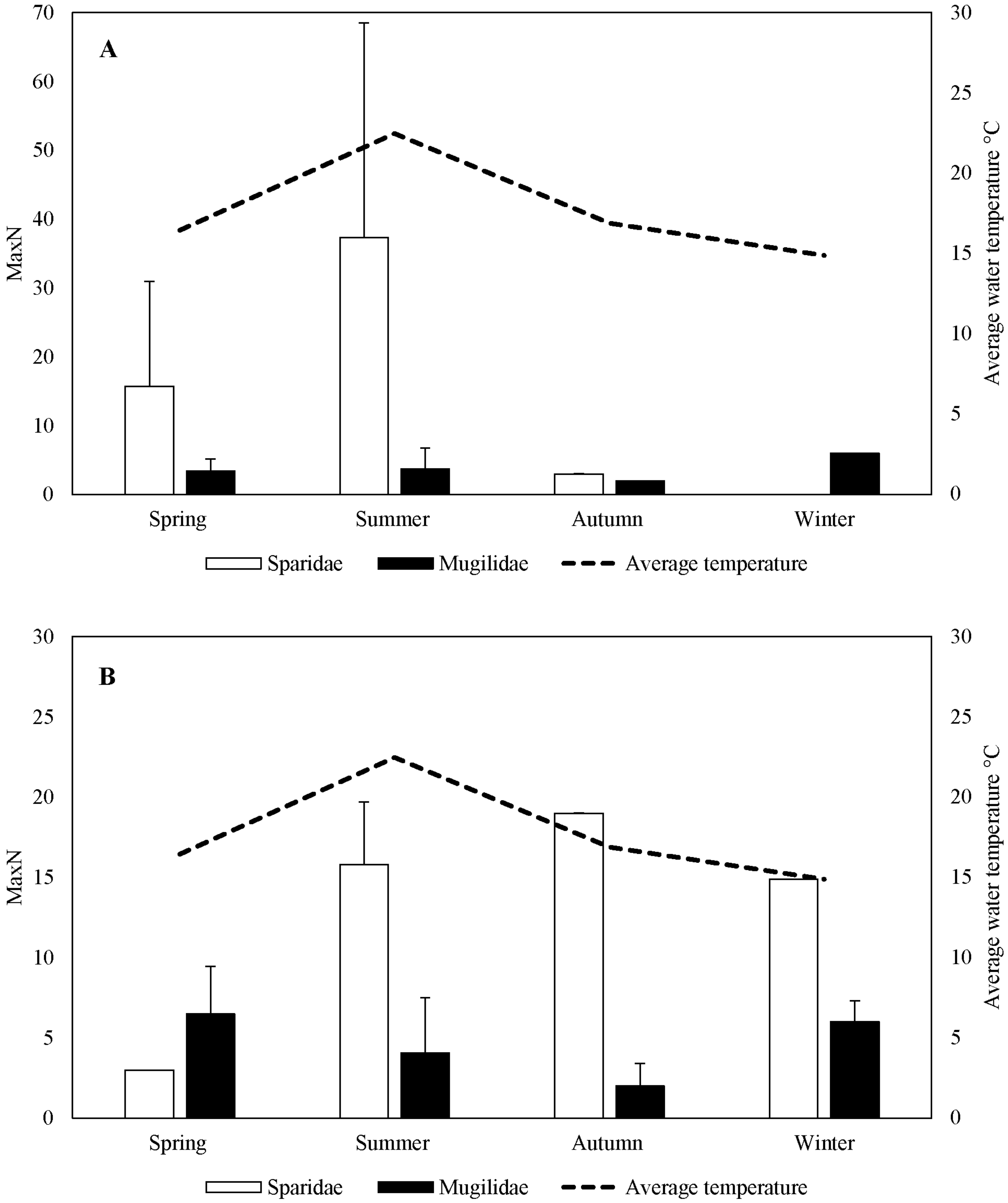

Figure 4.1: Average number of fish observed (+SD where possible) at *Zostera capensis* sites (A) and bare substratum sites (B) during four seasons.

When comparing the two sites it was noted that overall there were more Sparidae observed at Site 1 and more Mugilidae observed at Site 2. At Site 1 the highest MaxN observed belonged to the Sparidae in summer in the *Z. capensis* habitat (37.32 ± 31.12 SD) followed by the same family at the same habitat during spring (26.50 with no variability). The average number of Sparidae was also higher at the bare substratum habitats at Site 1 when compared to the average number of Mugilidae at both *Z. capensis* and bare substratum habitats at Site 1 (Figure 4.2 A). At Site 2 the highest MaxN of fish belonged to the Mugilidae in spring at the bare substratum habitat (7.52 ± 0.86 SD) followed by the same family at the *Z. capensis* habitat during summer (6.26 ± 2.99 SD). Sparidae were recorded at Site 2 in spring and winter on both *Z. capensis* and bare substratum habitats, but only at the bare substratum habitat in winter (Figure 4.2 B).

4.2. *Ulva* versus no *Ulva*

The Generalized Estimating Equations (GEE) used to run a Generalized Linear Model (GLM) on the *Ulva* versus no *Ulva* fish data (MaxN), which was limited to the winter periods in 2014 and 2015, showed that there was no significant difference between the fish family and year interaction ($\chi^2_{1.832}=1.072, p=0.300$), but there was a significant difference between the habitat and fish family interaction ($\chi^2_{1.832}=120.550, p<0.001$). The three-way interaction between habitat type, fish family and year also showed a significant difference ($\chi^2_{1.832}=13.159, p<0.001$) (Figure 4.3).

During August in both years the mean number of Sparidae was low at both the *Z. capensis* and bare substratum habitats. Significant differences can, however, be noted in the mean number of Mugilidae observed at the *Z. capensis* habitats under the two scenarios. During non-bloom conditions the mean number of Mugilidae associated with the *Z. capensis* was 0.54 (± 0.17 SE) (Figure 4.3), while under macroalgal bloom conditions the mean number was only 0.02.

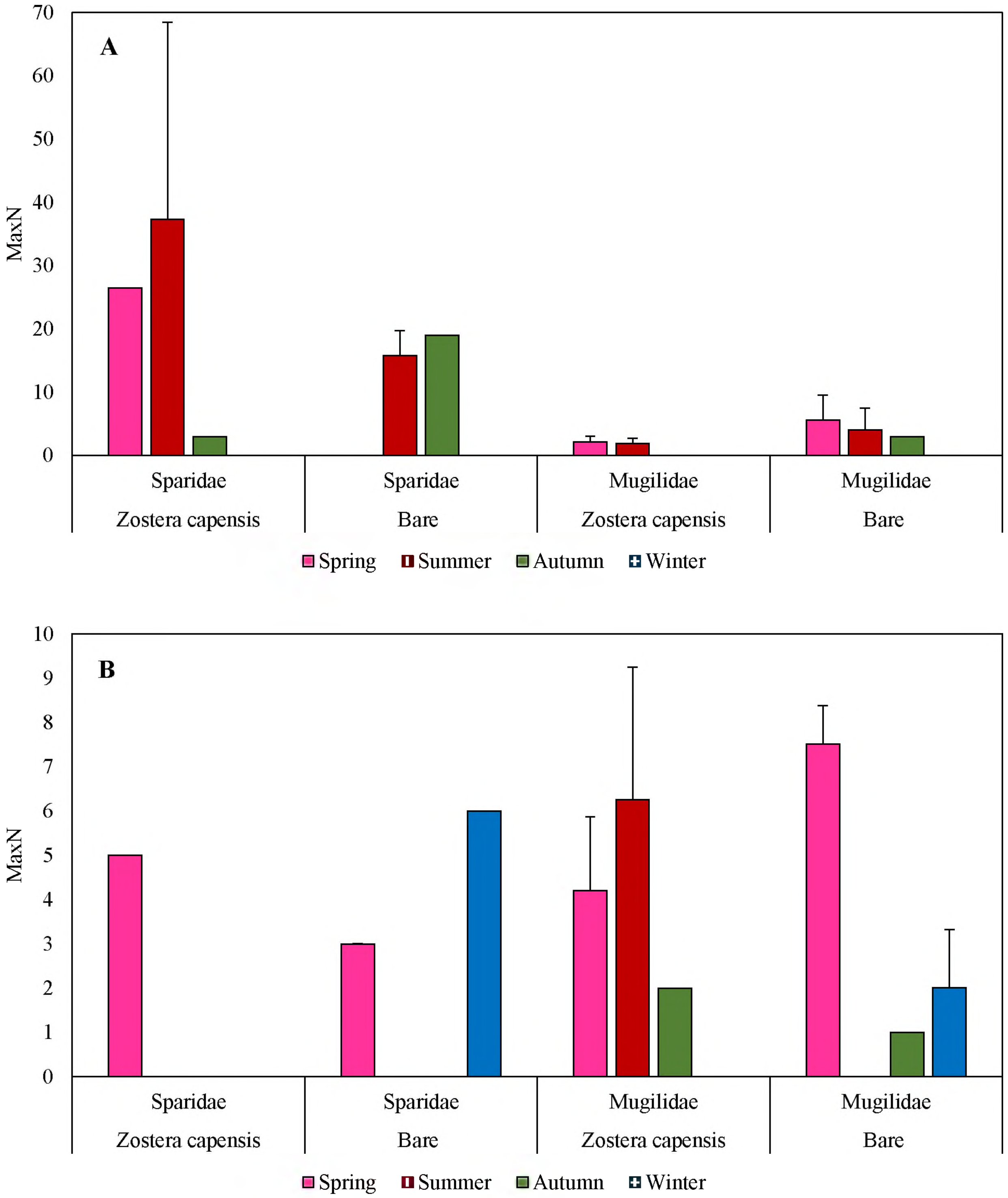

Figure 4.2: Average number of fish observed (+SD where possible) at *Zostera capensis* and bare substratum habitats at Site 1 (A) and Site 2 (B).

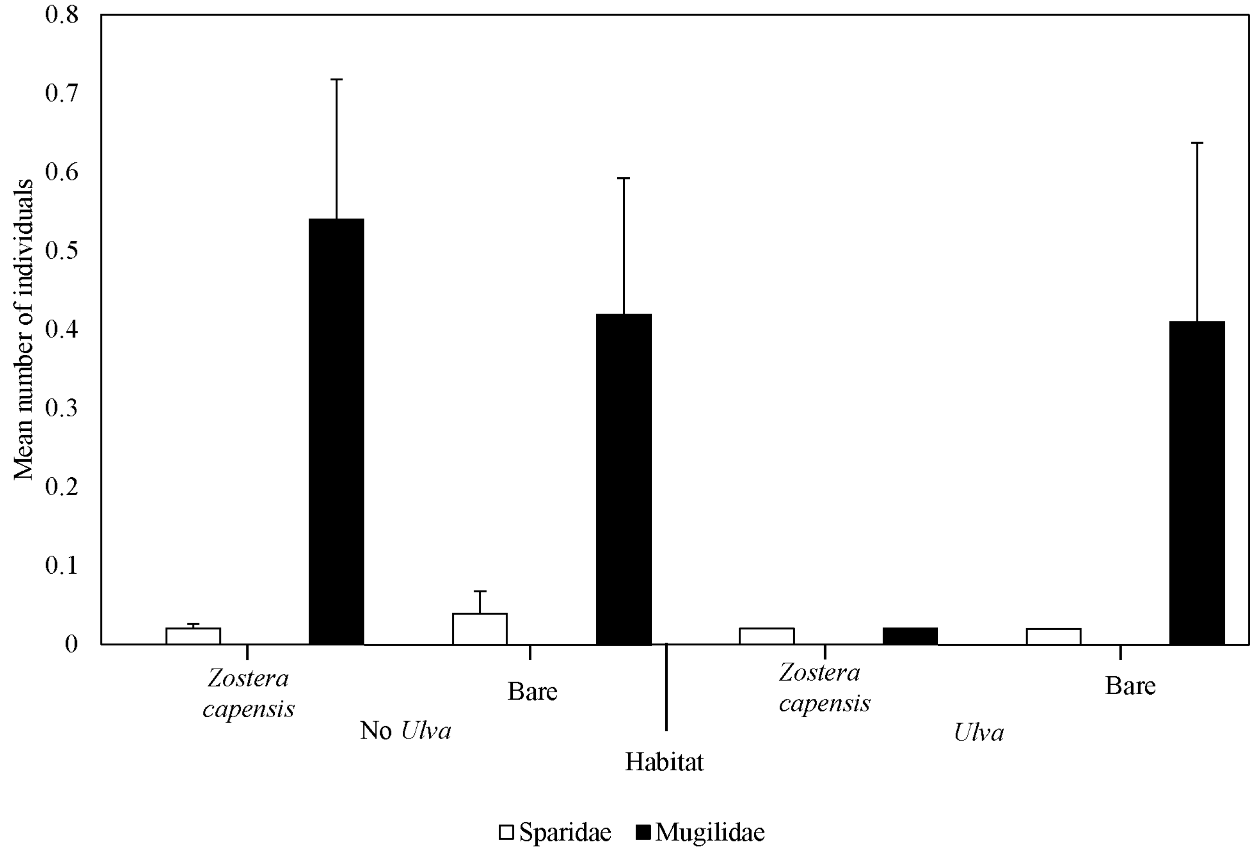

Figure 4.3: Mean number of fish (+SE where possible) for Mugilidae and Sparidae at *Zostera capensis* and bare substratum sites during non-bloom (2014) and *Ulva* bloom (2015) conditions.

4.3. Fish behaviour

Generalized Linear Models (GLM) analysis on behaviour data showed that there was no significant difference in the proportion of fish exhibiting feeding behaviour between habitats ($\chi^2_{1.16}$=0.001, p=0.974), families ($\chi^2_{1.16}$=0.034, p=0.855) or seasons ($\chi^2_{3.16}$=1.747, p=0.626). The interactions between habitat and family ($\chi^2_{2.16}$=0.040, p=0.980) and family and season ($\chi^2_{6.16}$=10.604, p=0.101) also showed no significant difference. At the bare substratum habitat both families exhibited a higher proportion of fish feeding behaviour in spring (4.75 ± 2.61 SE) and summer (4.00 ± 4.47 SE) compared to winter (1.75 ± 1.10 SE) and autumn (1.00 ± 1.41 SE). A similar pattern was recorded at the *Z. capensis* habitat, with summer (4.97 ± 2.70 SE) and spring (4.42 ± 4.25 SE) having a higher proportion of fish foraging when compared to winter (2.18 ± 3.03 SE) and autumn (1.24 ± 2.34 SE).

When comparing the two families, the same pattern prevailed. Mugilidae showed a higher proportion of feeding behaviour in spring (4.36 ± 2.10 SE) and summer (4.24 ± 2.89 SE), with lower proportion in winter (1.86 ± 1.72 SE) and autumn (1.06 ± 1.67 SE). Sparidae showed the highest levels of foraging overall (5.50 ± 4.23 SE) in summer followed by spring (4.89 ± 6.61 SE), with lower intensity feeding behaviour occurring during winter (2.41 ± 3.60 SE) and autumn (1.37 ± 2.70 SE).

Generalized Linear Models (GLM) using behaviour data showed that there was no significant difference in proportions of fish showing rapid swimming behaviour between habitats ($\chi^2_{1.36}$=0.701, p=0.403), families ($\chi^2_{1.36}$=0.210, p=0.647) or seasons ($\chi^2_{3.36}$=1.133, p=0.769). The interactions between habitat and family ($\chi^2_{1.36}$=0.341, p=0.559), habitat and season ($\chi^2_{1.36}$=1.100, p=0.294) and family and season ($\chi^2_{2.36}$=1.536, p=0.464) also showed no significant difference. At the bare substratum habitat, both families had higher proportions of fish showing rapid swimming behaviour in summer (2.50 ± 1.48 SE) and winter (1.12 ± 0.88 SE) than in spring (0.59 ± 0.68 SE), with no recording of this behaviour in autumn. The pattern changes at the *Z. capensis* habitat, with higher proportions of fish conducting this behaviour in summer (2.07 ± 0.95 SE) and spring (1.87 ± 1.37 SE) compared with autumn (1.39 ± 2.47 SE) or winter (no observations).

When comparing number of fish displaying rapid swimming in the two families, a slightly different pattern emerged. A higher proportion of Mugilidae showed rapid swimming behaviour in spring (2.45 ± 0.73 SE) followed by summer (1.83 ± 1.03 SE) and winter (1.25 ± 0.84 SE), with the lowest proportion recorded in autumn (1.00 ± 1.41 SE). Sparidae showed the highest overall proportion of rapid swimming behaviour (2.83 ± 1.39 SE) in summer followed by autumn (1.92 ± 4.97 SE), with a lower proportion fish showing rapid swimming behaviour in winter (1.00 ± 1.14 SE) and spring (0.45 ± 0.74 SE).

Generalized Linear Models (GLM) using behaviour data showed that there was no significant difference in the proportion of fish with slow meandering behaviour between habitats ($\chi^2_{1.37}$=0.185,

p=0.667), families ($\chi^2_{1.37}$=0.015, p=0.903) or seasons ($\chi^2_{3.37}$=2.540, p=0.468). The interactions between habitat and family ($\chi^2_{1.37}$=1.019, p=0.313), habitat and season ($\chi^2_{2.37}$=1.090, p=0.580) and family and season ($\chi^2_{2.37}$=0.575, p=0.750) also showed no significant difference. At the bare substratum habitat both families had higher proportions of slow meandering behaviour in summer (3.97 ± 2.00 SE), followed by spring (1.37 ± 0.86 SE) and autumn (1.00 ± 1.00 SE), with the lowest proportions of this behaviour recorded in winter (0.80 ± 1.20 SE). The pattern changed slightly at the *Z. capensis* habitat with fish in spring (1.95 ± 0.95 SE) and summer (1.91 ± 0.97 SE) showing higher proportions, lower proportions in autumn (0.57 ± 1.03 SE) and no recordings of slow meandering behaviour in winter.

When comparing the two families, the Mugilidae exhibited slow meandering behaviour in higher proportions than the Sparidae. Mugilidae showed the highest proportions of fish with slow swimming behaviour overall (2.83 ± 1.51 SE) in summer and spring (2.43 ± 0.72 SE), with winter (1.20 ± 0.97 SE) and autumn (0.57 ± 1.03 SE) having lower proportions. Sparidae had higher proportions of fish showing rapid swimming behaviour in summer (2.67 ± 1.28 SE) followed by spring (1.10 ± 0.90 SE), autumn (1.00 ± 1.00 SE) and winter (0.43 ± 1.19 SE).

4.4. Underwater visual monitoring versus seine netting

The proportion of different fish species observed in the camera footage was compared to the proportion of different fish species caught in the seine net. A Spearman's rank correlation data analysis revealed a significant weak positive linear correlation ($r_s = 0.465$, $p<0.001$) between the proportion of fish species observed on video and the proportion of fish species caught by the seine net (Figure 4.4 A). Fish species observed in camera footage and caught by the seine net were mostly similar, however some species observed in video footage were not caught by the seine net. Similarly, some of the fish species caught by the seine net were not observed in the video footage (Figure 4.5).

A Cook's Distance test was used to check for influence points that might change the outcome of the Spearman's rank correlation as on one day of sampling both the video footage and seine net captured a large number of sparids. Two influence points were identified, one for *Diplodus hottentotus* and one for *Sarpa salpa*. These two points were removed from the data set and the Spearman's rank correlation was run again. The analysis still revealed a significant weak positive correlation although a little stronger than before ($r_s = 0.480$, $p<0.001$) and the R^2 value changed slightly from 0.1054 to 0.1009 (Figure 4.4 B).

The video analysis showed that during upwelling conditions (12, 13, 26 and 27 November 2014), when water temperatures were between 11°C and 16°C, the number of sparid species seen was very low. The fish most observed during cool water conditions were mullet species, pooled together as mugilids, as well as the evil-eyed puffer (*Amblyrhynchotes honckenii*) (Figure 4.5 A). As the water temperature increased to between 21°C and 23°C (28 and 29 January 2015, 10 and 11 February 2015), there was a substantial increase in sparids such as *Sarpa salpa* and *Diplodus capensis*, and to a lesser extent *Rhabdosargus holubi*. Mugilidae were still recorded on the camera footage but comprised a much smaller percentage of observed fish (Figure 4.5 A). The seine netting analysis showed a similar trend to the camera footage. During cool water temperatures, the fish caught in the net consisted of mugilids and *A. honckenii* (Figure 4.5 B). As soon as the water temperature increased, large numbers of sparids were captured, including *S. salpa*, *D. capensis* and *R. holubi* (Figure 4.5 B).

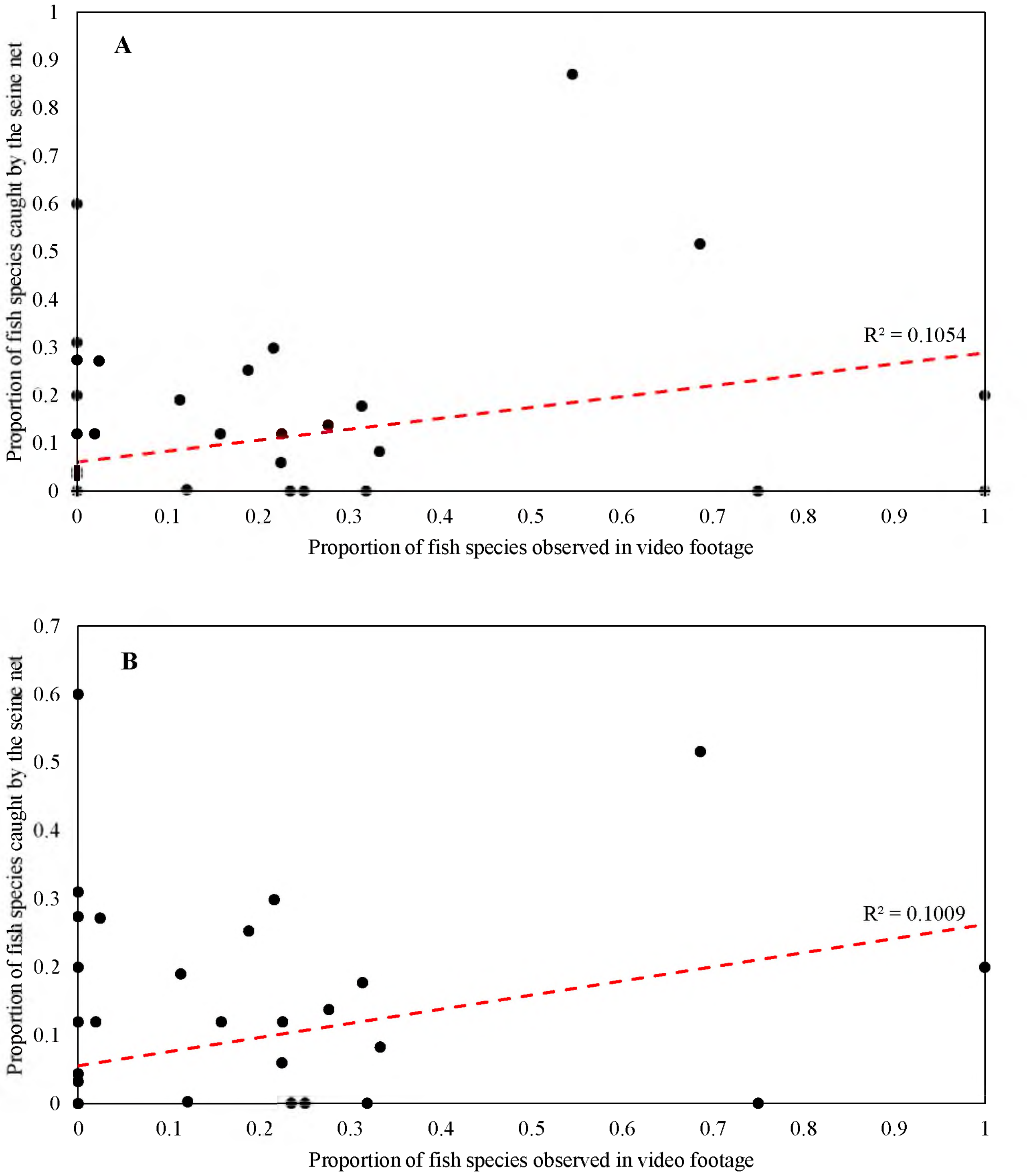

Figure 4.4: Scatterplot of proportional video sampling data and proportional seine net sampling data with the whole data set (A) and data after removing two influence points identified by a Cook's Distance test (B).

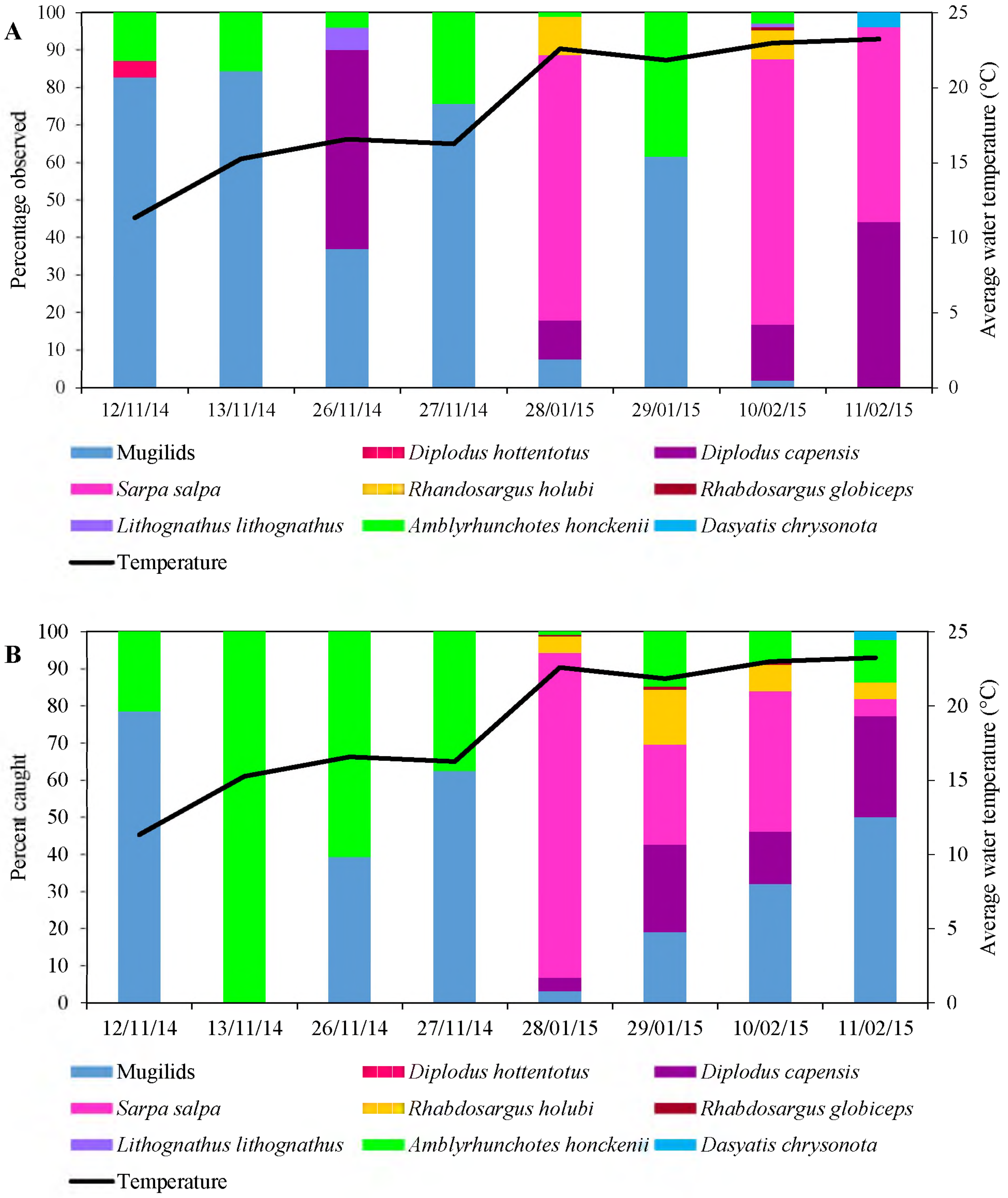

Figure 4.5: Percentage of fish observed on video footage (A) and caught by seine net sampling (B) during upwelling (12, 13, 26, 27 November) and non-upwelling conditions (28, 29 January and 10, 11 February).

4.5. Historical data

As there were three factors to consider, namely habitat (sparse *Z. capensis*, dense *Z. capensis* and bare substratum), locality (bay, lagoon and estuary) and season (spring, summer, autumn and winter), the statistical package multiple Analysis of Similarities (ANOSIM) was applied to the data. The first two-way crossed analysis run on locality and habitat data showed a significant difference between localities across all habitats ($R=0.140$, $p<0.05$) as well as a significant difference between habitats across all localities ($R=0.068$, $p<0.05$).

The second two-way crossed analysis run on seasonal and habitat data showed a significant difference between seasons across all habitats ($R=0.135$, $p<0.05$), as well as a significant difference between habitats across all seasons ($R=0.123$, $p<0.05$). The third two-way crossed analysis run on seasonal and locality data showed a significant difference between seasons across all sites ($R=0.156$, $p<0.05$), as well as a significant difference between localities across all seasons ($R=0.221$, $p<0.05$).

The non-metric Multi-Dimensional Scaling (MDS) analysis run on the habitats (Figure 4.6), localities (Figure 4.7) and seasonal data (Figure 4.8) had high stress levels (0.22) and showed no clear separation in the data set, although there are some outliers. There are a noticeably larger number of sparse and dense *Zostera* data points than bare sediment data points in the habitat plot (Figure 4.6) and there are also a much smaller number of estuary data points compared to bay and lagoon data points (Figure 4.7). These differences in the amount of data points can affect the results as there are less bare data points to compare to the *Z. capensis* data points and thus will not give a complete representation of the bare sediment habitat. The same will be seen for the estuary data as there are less data points for that locality and thus will provide a less complete picture when compared to bay and lagoon.

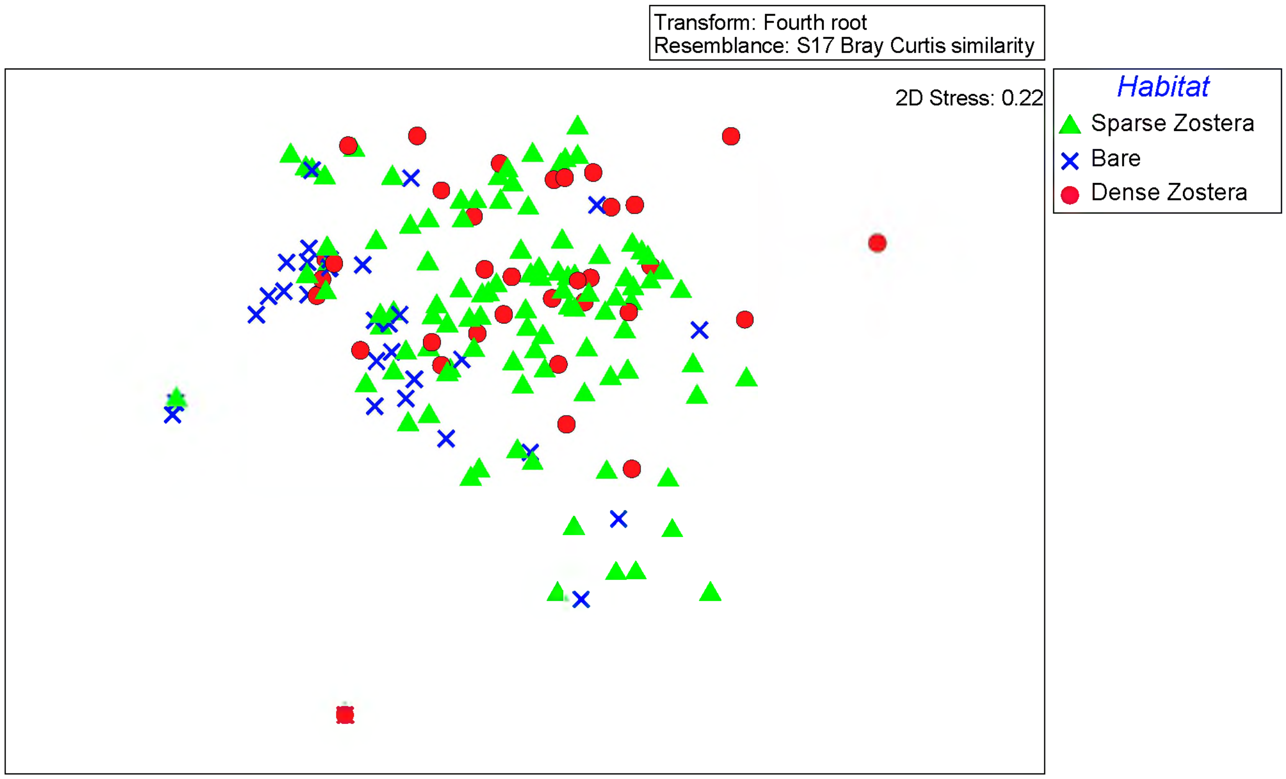

Figure 4.6: Results from the non-metric Multi-Dimensional Scaling (MDS) performed on the habitat data.

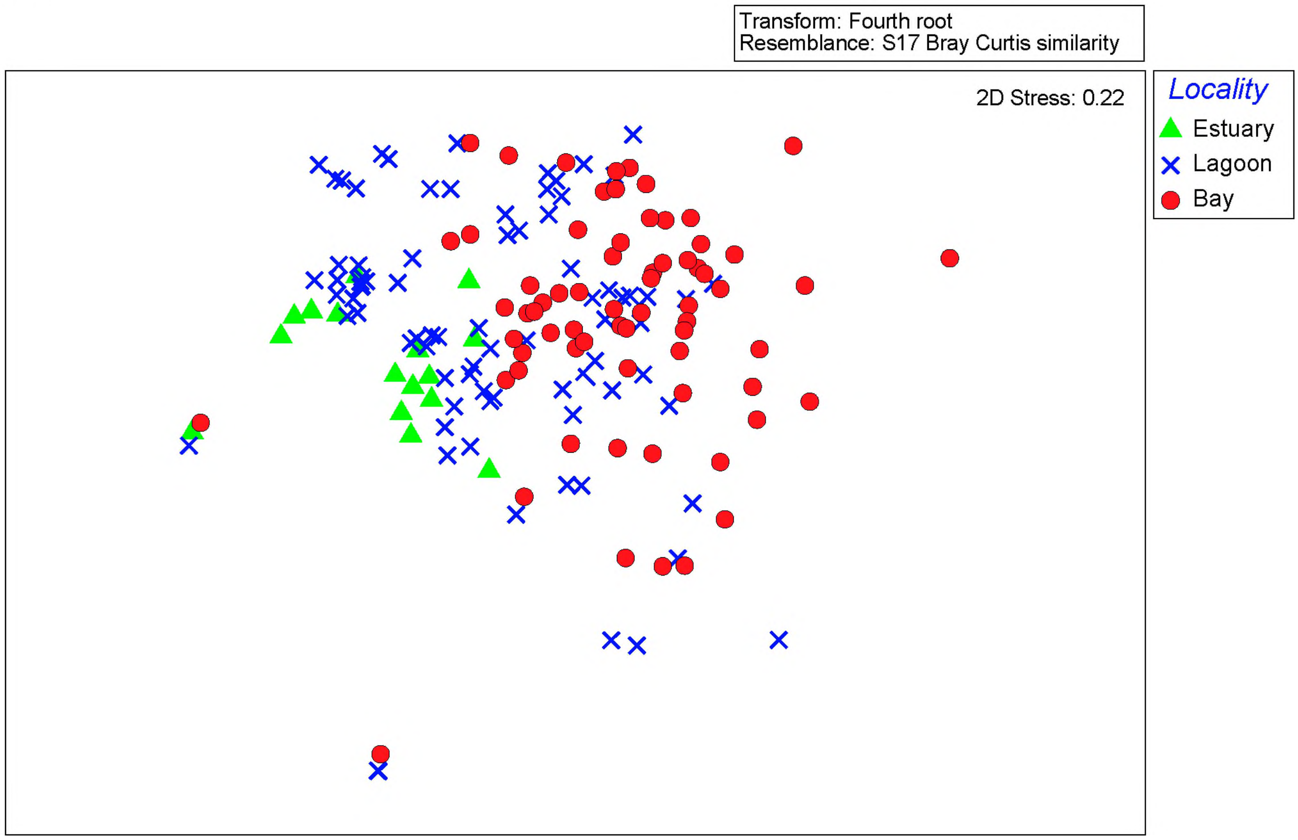

Figure 4.7: Results from the non-metric Multi-Dimensional Scaling (MDS) performed on the locality data.

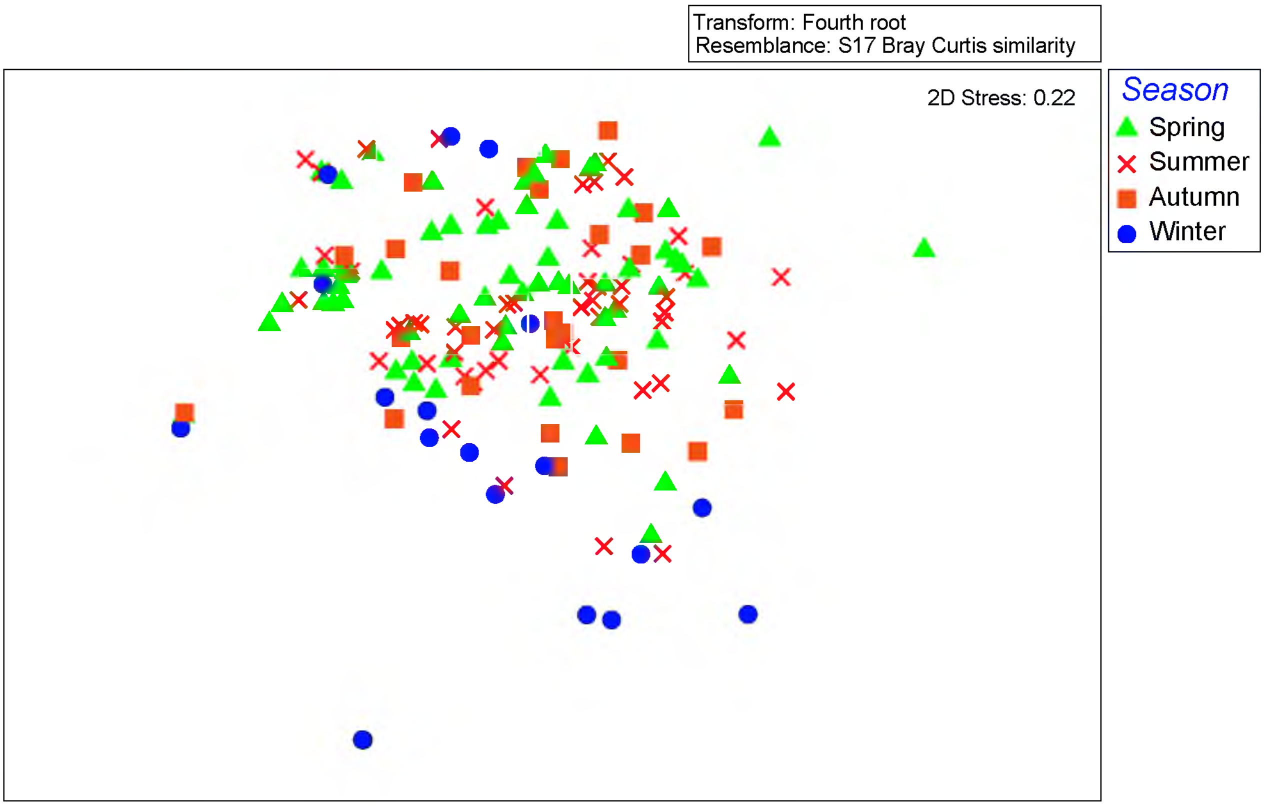

Figure 4.8: Results from the non-metric Multi-Dimensional Scaling (MDS) performed on the season data.

The two-way Similarity Percentages (SIMPER) analysis comparing habitats across all localities indicated relatively low within group similarities for bare sediment (38.17%) with slightly higher similarities for dense *Z. capensis* (40.59%) and sparse *Z. capensis* (43.28%). The species that contributed most to the within group similarity for bare sediment were *Rhabdosargus holubi* (55.94%) and *Lithognathus lithognathus* (30.70%). For dense *Z. capensis R. holubi* (40.07%) and *Diplodus capensis* (17.35%) contributed the most to within group similarities while for sparse *Z. capensis* it was again *R. holubi* (41.42%) followed by *Liza richardsonii* (16.49%) (Table 4.1).

The average dissimilarity between sparse *Z. capensis* and bare substratum was 62.69%. Nine species cumulatively contributed > 90% of the dissimilarity with *R. holubi* (23.51%) and *L. richardsonii* (14.76%) contributing the most. The average dissimilarity between sparse and dense *Z. capensis* was less (58.26%) and 11 species cumulatively contributed > 90% of the dissimilarity which included *R. holubi* (13.17%) and *S. salpa* (13.14%). The average dissimilarity between bare substratum and dense *Z. capensis* was the highest (63.27%) with nine species cumulatively contributing > 90% which included *R. holubi* (20.16%) and *L. dumerili* (14.12%) For the dissimilarity we see other species also contributing towards the dissimilarity that are not as abundant as the ones that have been mentioned. These species *include Myxus capensis, Mugil cephalus, Spondyliosoma emarginatum, Lithognathus mormyrus, Liza tricuspidens, Diplodus hottentotus* and *Crenidens crenidens* (Table 4.2).

Table 4.1: Contribution (%) of the dominant sparid and mugilid species to the within group similarity for bare sediment, dense and sparse *Zostera capensis* data across all localities.

	Habitat		
Species	Bare	Sparse *Z. capensis*	Dense *Z. capensis*
Rhabdosargus holubi	55.94	41.42	40.07
Liza richardsonii	10.57	16.49	7.90
Lithognathus lithognathus	30.70	0	6.46
Diplodus capensis	0	15.78	17.35
Sarpa salpa	0	8.64	14.79
Liza dumerili	0	7.73	7.19

Table 4.2: Contribution (%) of the dominant sparid and mugilid species to the between group dissimilarity for bare sediment, dense and sparse *Zostera capensis* data across all localities.

	Habitat		
Species	Sparse *Z. capensis* and Bare sediment	Sparse *Z. capensis* and Dense *Z. capensis*	Bare sediment and Dense *Z. capensis*
Rhabdosargus holubi	23.51	13.17	20.16
Liza dumerili	13.13	11.96	14.12
Liza richardsonii	14.76	11.14	11.89
Lithognathus lithognathus	13.72	9.21	12.59
Sarpa salpa	6.14	13.14	10.57
Diplodus capensis	10.77	12.08	9.44
Diplodus hottentotus	3.56	4.62	5.94
Lithognathus mormyrus	3.25	5.78	3.75
Mugil cephalus	0	4.13	3.70
Spondyliosoma emarginatum	0	3.55	0
Liza tricuspidens	0	3.43	0
Myxus capensis	3.28	0	0

When comparing the locality data across all habitats, a low similarity level for the lagoon regime (40.44%), a slightly higher similarity level for the bay regime (44.69%) and the highest similarity for the estuary regime (57.75%) was indicated. For the bay data, the species that contributed most to the within group similarity was *D. capensis* (23.89%) and *L. richardsonii* (20.60%). For the estuary there were only three species contributing to the within group similarity with *L. lithognathus* contributing the most (52.44%). For the lagoon, *R. holubi* contributed to more than half of the within group similarity (61.47%) followed by *L. richardsonii* (10.80%) (Table 4.3).

The average dissimilarity between estuary and lagoon was 56.20%. Eight species cumulatively contributed > 90% of the dissimilarity with *L. lithognathus* (28.18%) and *R. holubi* (19.67%) contributing the most. The average dissimilarity between estuary and bay was the highest (62.89%) and eight species cumulatively contributed > 90% of the dissimilarity which included *L. lithognathus* (16.16%) and *D. capensis* (15.21%). The average dissimilarity between lagoon and bay was slightly lower (62.82%) with 10 species cumulatively contributing > 90% which included *S. salpa* (14.76%) and *D. capensis* (14.75%) (Table 4.4)

Table 4.3: Contribution (%) of the dominant sparid and mugilid species to the within group similarity for bay, estuary and lagoon data across all habitats.

	Locality		
Species	Bay	Lagoon	Estuary
Rhabdosargus holubi	21.85	61.47	36.17
Lithognathus lithognathus	3.89	8.90	52.44
Diplodus capensis	23.89	7.05	0
Liza richardsonii	20.60	10.80	9.49
Sarpa salpa	16.39	0	0
Liza dumerili	6.76	7.84	0

Table 4.4: Contribution (%) of the dominant sparid and mugilid species to the between group dissimilarity for bay, estuary and lagoon data across all habitats.

Species	Estuary and Lagoon	Estuary and Bay	Lagoon and Bay
Lithognathus lithognathus	28.18	16.16	8.32
Rhabdosargus holubi	19.67	13.91	12.72
Liza richardsonii	16.40	9.21	11.81
Diplodus capensis	6.30	15.21	14.75
Sarpa salpa	3.10	14.21	14.76
Liza dumerili	9.61	9.81	10.78
Myxus capensis	5.78	3.59	0
Spondyliosoma emarginatum	0	3.59	5.15
Lithognathus mormyrus	0	0	6.07
Liza tricuspidens	0	0	3.84
Diplodus hottentotus	0	0	3.46
Mugil cephalus	3.02	0	0

The two-way SIMPER analysis comparing habitats across all seasons indicated low within group similarities for dense *Z. capensis* (39.90%), with slightly higher similarity levels for bare sediment (43.77%) and sparse *Z. capensis* (43.95%). There were only two species that contributed to the within group similarity for bare sediment, namely *R. holubi* (53.88%) and *L. lithognathus* (37.14%). *Rhabdosargus holubi* (42.55%) contributed most to the within group similarity for sparse *Z. capensis* followed by *L. richardsonii* (16.09%). In dense *Z. capensis*, *R. holubi* once again contributed the most to the within group similarity (43.15%) followed by *D. capensis* (11.78%) (Table 4.5).

The average dissimilarity between sparse *Z. capensis* and bare substratum was 63.29%. Nine species cumulatively contributed > 90% of the dissimilarity with *L. lithognathus* (15.92%) and *D. capensis* (14.56%) contributing the most. The average dissimilarity between sparse and dense *Z. capensis* was less (58.19%) and 11 species cumulatively contributed > 90% of the dissimilarity which included *S. salpa* (13.17%) and *D. capensis* (13.38%). The average dissimilarity between bare substratum and dense *Z. capensis* was the highest (64.33%) with nine species cumulatively contributing > 90% which included *R. holubi* (16.13%) and *L. lithognathus* (14.50%) (Table 4.6).

Table 4.5: Contribution (%) of the dominant sparid and mugilid species to the within group similarity for bare sediment, dense and sparse *Zostera capensis* data across all seasons.

	Habitat		
Species	Bare	Sparse *Z. capensis*	Dense *Z. capensis*
Rhabdosargus holubi	53.88	42.55	43.15
Lithognathus lithognathus	37.14	5.85	9.05
Liza richardsonii	0	16.09	9.63
Diplodus capensis	0	15.99	11.78
Sarpa salpa	0	8.11	11.52
Liza dumerili	0	7.12	6.80

Table 4.6: Contribution (%) of the dominant sparid and mugilid species to the between group dissimilarity for bare sediment, dense and sparse *Zostera capensis* data across all seasons.

Species	Sparse *Z. capensis* and Bare sediment	Sparse *Z. capensis* and Dense *Z. capensis*	Bare sediment and Dense *Z. capensis*
Rhabdosargus holubi	13.65	12.22	16.13
Lithognathus lithognathus	15.92	9.64	14.50
Sarpa salpa	12.46	14.57	14.36
Diplodus capensis	14.56	13.38	12.62
Liza richardsonii	14.15	10.83	11.14
Liza dumerili	9.99	10.98	10.07
Lithognathus mormyrus	4.09	5.83	4.63
Diplodus hottentotus	3.26	4.42	3.00
Mugil cephalus	0	4.49	5.58
Myxus capensis	3.69	0	0
Spondyliosoma emarginatum	0	3.21	0
Liza tricuspidens	0	3.04	0

When comparing seasons across all habitats, the highest similarity levels were indicated for spring (45.98%) followed by summer (44.33%), with autumn showing slightly lower similarity levels (40.05%) and winter the lowest (18.78%). For autumn the species that contributed most to the within group similarity was *R. holubi* (39.48%) and *D. capensis* (15.98%). For spring, *R. holubi* contributed the most to the within group similarity (48.43%) followed again by *D. capensis* (18.34%). For summer, *R. holubi* (38.69%) once again contributed the most to the within group similarity followed by *L. richardsonii* (21.42%). In winter *L. richardsonii* (54.53%) and *R. holubi* (26.79%) contributed most to within group similarity (Table 4.7).

The average dissimilarity between spring and summer was 55.55%. Ten species cumulatively contributed > 90% of the dissimilarity with *S. salpa* (14.23%) and *D. capensis* (13.54%) contributing the most. The average dissimilarity between spring and autumn was higher (57.79%) and 10 species cumulatively contributed > 90% of the dissimilarity which included *S. salpa* (13.20%) and *D. capensis* (12.98%). The average dissimilarity between summer and autumn was 57.23% with 11 species cumulatively contributing > 90% which included *D. capensis* (12.73%) and *L. dumerili* (12.63%). The average dissimilarity between spring and winter was the highest (74.18%) with eight species cumulatively contributing > 90% which included *R. holubi* (24.49%) and *L. lithognathus* (15.66%). The average dissimilarity between summer and winter was 71.30% with nine species cumulatively contributing > 90% which included *R. holubi* (21.78%) and *L. richardsonii* (13.23%). The average dissimilarity between autumn and winter was the second highest (72.55%) with nine species cumulatively contributing > 90% which included *R. holubi* (19.68%) and *L. dumerili* (14.14%) (Table 4.8).

Table 4.7: Contribution (%) of the dominant sparid and mugilid species to the within group similarity for all four seasons across all habitats.

	Season			
Species	Autumn	Spring	Summer	Winter
Rhabdosargus holubi	39.48	48.43	38.69	26.79
Liza richardsonii	13.21	8.95	21.42	54.53
Diplodus capensis	15.98	14.81	13.12	0
Lithognathus lithognathus	10.67	9.91	7.56	8.07
Liza dumerili	11.76	0	8.74	7.18
Sarpa salpa	0	10.50	4.70	0

Table 4.8: Contribution (%) of the dominant sparid and mugilid species to the between group dissimilarity for all four seasons across all habitats.

Species	Spring and Summer	Spring and Autumn	Summer and Autumn	Spring and Winter	Summer and Winter	Autumn and Winter
Rhabdosargus holubi	11.24	11.61	11.58	24.49	21.78	19.68
Lithognathus lithognathus	9.81	9.72	9.18	15.66	12.93	12.82
Sarpa salpa	14.23	13.20	10.25	11.34	8.33	7.07
Liza richardsonii	12.29	10.87	11.27	13.43	13.23	12.64
Diplodus capensis	13.54	12.98	12.73	11.46	12.80	11.82
Liza dumerili	10.76	12.64	12.63	8.54	10.62	14.14
Lithognathus mormyrus	6.18	5.89	6.36	3.34	4.43	4.07
Spondyliosoma emarginatum	4.64	6.14	7.18	0	3.22	4.70
Liza tricuspidens	3.47	4.48	5.46	0	2.87	4.01
Diplodus hottentotus	4.58	3.32	3.18	0	0	0
Crenidens crenidens	0	0	2.85	0	0	0
Mugil cephalus	0	0	0	2.69	0	0

The two-way SIMPER analysis comparing localities across all seasons indicated the lowest within group similarity for the lagoon regime (43.78%) followed by the bay regime (46.67%), with the estuary regime showing high levels of within group similarity (60.36%). For the bay regime, the species that contributed most to within group similarity were *D. capensis* (25.84%) and *R. holubi* (22.25%). For the estuary regime there were only two species contributing to the within group similarity, with *L. lithognathus* contributing the most (57.29%). For the lagoon regime, *R. holubi* contributed more than half of the within group similarity (63.76%) followed by *L. lithognathus* (10.21%) (Table 4.9).

The average dissimilarity between estuary and lagoon was 57.15%. Eight species cumulatively contributed > 90% of the dissimilarity with *L. lithognathus* (24.49%) and *R. holubi* (18.53%) contributing the most. The average dissimilarity between estuary and bay was the highest (69.95%) and nine species cumulatively contributed > 90% of the dissimilarity which included *L. lithognathus* (17.13%) and *S. salpa* (16.77%). The average dissimilarity between lagoon and bay was slightly lower (62.00%) with 10 species cumulatively contributing > 90% which included *S. salpa* (16.35%) and *D. capensis* (15.64%) (Table 4.10).

Table 4.9: Contribution (%) of the dominant sparid and mugilid species to the within group similarity for bay, estuary and lagoon data across all seasons.

	Locality		
Species	Bay	Lagoon	Estuary
Rhabdosargus holubi	22.25	63.76	35.38
Lithognathus lithognathus	3.80	0	57.29
Diplodus capensis	25.84	5.60	0
Liza richardsonii	16.30	9.71	0
Sarpa salpa	19.13	0	0
Liza dumerili	5.74	6.29	0

Table 4.10: Contribution (%) of the dominant sparid and mugilid species to the between group dissimilarity for bay, estuary and lagoon data across all seasons.

Species	Estuary and Lagoon	Estuary and Bay	Lagoon and Bay
Lithognathus lithognathus	24.49	17.13	8.70
Rhabdosargus holubi	18.53	10.81	11.91
Sarpa salpa	5.92	16.77	16.35
Diplodus capensis	8.75	16.17	15.64
Liza richardsonii	14.86	10.53	11.30
Liza dumerili	9.45	7.20	10.11
Lithognathus mormyrus	0	4.89	5.99
Myxus capensis	5.66	3.32	0
Mugil cephalus	3.47	3.45	3.27
Spondyliosoma emarginatum	0	0	4.03
Diplodus hottentotus	0	0	3.69

When comparing seasons across all localities, similar similarity levels were indicated for summer (47.89%) and spring (47.13%) followed by autumn (41.66%), with the lowest similarity levels recorded during winter (15.29%). For autumn the species that contributed most to the within group similarity were *R. holubi* (39.10%) and *D. capensis* (17.42%). Spring showed the same pattern, with *R. holubi* contributing the most to the within group similarity (51.66%) followed by *D. capensis* (12.12%). For summer, *R. holubi* (40.99%) and *L. richardsonii* (16.71%) contributed the most to the within group similarity and the same in winter, with *L. richardsonii* (48.08%) and *R. holubi* (32.86%) contributing the most to within group similarity (Table 4.11).

The average dissimilarity between spring and summer was 52.77%. Eleven species cumulatively contributed > 90% of the dissimilarity with *L. richardsonii* (12.68%) and *L. dumerili* (12.42%) contributing the most. The average dissimilarity between spring and autumn was higher (55.41%) and 11 species cumulatively contributed > 90% of the dissimilarity which included *L. dumerili* (13.94%) and *R. holubi* (12.01%). The average dissimilarity between summer and autumn was 54.43% with 11species cumulatively contributing > 90% which included *L. dumerili* (13.38%) and *D. capensis* (12.30%). The average dissimilarity between spring and winter was the second highest (75.48%) with eight species cumulatively contributing > 90% which included *R. holubi* (29.03%) and *L. lithognathus* (13.21%). The average dissimilarity between summer and winter was 74.27% with nine species cumulatively contributing > 90% which included *R. holubi* (28.02%) and *L. richardsonii* (14.28%). The average dissimilarity between autumn and winter was the highest (76.58%) with nine species cumulatively contributing > 90% which included *R. holubi* (23.37%) and *L. dumerili* (14.55%) (Table 4.12).

Table 4.11: Contribution (%) of the dominant sparid and mugilid species to the within group similarity for all four seasons across all localities.

	Season			
Species	Autumn	Spring	Summer	Winter
Rhabdosargus holubi	39.10	51.66	40.99	32.86
Liza richardsonii	13.13	8.01	16.71	48.08
Diplodus capensis	17.42	12.12	15.17	5.32
Lithognathus lithognathus	6.92	10.30	7.13	8.88
Sarpa salpa	7.33	10.49	6.21	0
Liza dumerili	10.21	0	7.95	0

Table 4.12: Contribution (%) of the dominant sparid and mugilid species to the between group dissimilarity for all four seasons across all localities.

Species	Spring and Summer	Spring and Autumn	Summer and Autumn	Spring and Winter	Summer and Winter	Autumn and Winter
Rhabdosargus holubi	12.18	12.01	11.30	29.03	28.02	23.37
Liza dumerili	12.42	13.94	13.38	10.02	12.02	14.55
Liza richardsonii	12.68	10.38	10.42	13.16	14.28	11.43
Lithognathus lithognathus	10.93	9.27	8.69	13.21	11.42	10.57
Diplodus capensis	11.67	12.91	12.30	10.35	9.05	12.40
Sarpa salpa	11.79	11.51	10.12	9.35	6.19	6.91
Lithognathus mormyrus	5.75	5.91	6.38	2.92	3.55	3.97
Diplodus hottentotus	5.44	3.29	4.17	2.80	4.33	0
Spondyliosoma emarginatum	3.53	6.27	7.11	0	0	5.19
Liza tricuspidens	0	3.80	4.79	0	2.79	2.97
Mugil cephalus	3.22	2.94	0	0	0	0
Crenidens crenidens	0	0	3.64	0	0	0
Myxus capensis	3.53	0	0	0	0	0

Chapter 5 - Discussion

There are a number of factors that influence the abundance of marine fish in South African estuaries, including predation, turbidity, habitat variation, whether an estuary is open or closed, estuary size, the occurrence and severity of floods, the ability of species to adjust to temperature and salinity fluctuations and habitat degradation (Whitfield and Kok, 1992; Whitfield, 2016). These factors will also have a great impact on the important nursery function that estuaries provide for numerous fish species of which many are commercially important (Wallace *et al.*, 1984; Bennett, 1989; Gladstone *et al.*, 2012; Whitfield and Pattrick, 2015).

Seagrass meadows can be found in the shallow coastal waters of most continents (Whitfield *et al.*, 1989; Heck *et al.*, 2003; Whitfield, 2016). These habitats have many valuable functions that include reducing the impact of wave action on shorelines, sediment stabilization, detrital production and export, increasing dissolved oxygen concentrations of estuarine waters, nutrient recycling, provision of optimal habitat for growth, survival and reproduction of a diverse range of invertebrate and vertebrate taxa and the role they play as a nursey for an array of juvenile fish of which many species are commercially and recreationally important (Pollard, 1984; Wallace *et al.*, 1984; Bennett, 1989; Whitfield *et al.*, 1989; Sogard and Able, 1991; Short and Wyllie-Echeverria, 1996; Beck *et al.*, 2001; Heck *et al.*, 2003; Whitfield, 2016). Seagrass meadows are undoubtedly important for many fish and it is therefore important to fully understand the utilization of these habitats by different fish species.

5.1. Underwater visual monitoring

The underwater visual monitoring data showed that there was no significant difference in the number of Mugilidae and Sparidae seen on the video footage overall, although there was a significant difference in the number of fish seen from the two families in two different habitats, *Z. capensis* and bare substratum. Mugilidae were significantly more abundant at bare substratum sites and, although not statistically significant, there was a trend of more Sparidae at *Z. capensis* sites. Although no statistical analysis could be done on the seasonal aspect of the data set, graphical representation showed that Sparidae were most abundant at *Z. capensis* sites during spring and summer months, while Mugilidae were most abundant at bare substratum sites during spring and winter. When the two sampling sites were compared, Sparidae were more abundant at Site 1 and Mugilidae more abundant at Site 2. This could be due to the fragmentation of the different sites as Site 1 consisted of smaller fragmented areas of *Z. capensis* and bare substratum, while Site 2 consisted of a clear separation between the *Z. capensis* site and the bare substratum site with almost no fragmentation occurring. Habitat edges can alter physical conditions, species interactions and resource distributions and so affect species distributions and abundances (Smith *et al.*, 2011). As an increase in smaller patches of seagrass increases the edge-to-area ratio, fragmentation of these habitats can lead to positive edge effects and so support greater abundances of fish (Macreadie *et al.*, 2009).

Many authors have concluded that when unvegetated areas are compared to vegetated areas in both oceanic and estuarine habitats, the latter have higher fish densities, abundance and diversity (Pollard, 1984; Sogard and Able, 1991; Adams, 2016; Whitfield, 2016). Dibble *et al.* (1996) found up to seven times more fish in vegetated areas than in unvegetated areas. Results from these studies have also shown that as plant density increases, smaller and younger fishes are more abundant. Both Whitfield *et al.* (1989) and Dibble *et al.* (1996) found that when vegetated and unvegetated habitats in the same water body are compared, the latter generally has fewer species and lower densities of fishes. The findings of the current study supports the results of both Whitfield *et al.* (1989) and Dibble *et al.* (1996) as there was a higher abundance and diversity of fishes associated with *Z. capensis* habitats when compared to the adjacent bare substratum habitats in the Knysna system.

Submerged macrophytes are known to provide protection for small fishes from predation (Pollard, 1984; Sogard and Able, 1991); however, according to Whitfield (2016) the type of macrophyte habitat and its complexity will determine the level of protection. A good example of this is a study on the sparid *Rhabdosargus holubi* which showed that although this species was most abundant in *Z. capensis* beds in the Bushmans Estuary (South Africa), it did not feed solely in this habitat, thus indicating that the protection provided by the *Z. capensis* was the main reason this species is associated with that specific habitat (Leslie, 2015). The same fish species was found to be very abundant in Z. capensis habitats in the current study using underwater video monitoring and seine netting at the same sites, as well as in the historical seine netting data sets.

The horizontal location of seagrass beds can play a significant role in the colonisation of this habitat (Ter Morshuizen and Whitfield, 1994; Whitfield, 2016). In Australia, early juveniles of marine migrants settle in seagrass beds closest to the mouth of an estuary, whereas larger specimens of the same species are found in seagrass beds higher up the estuary (Hannan and Williams, 1998). Abundances of fishes associated with seagrass beds also follow the cyclical growth pattern of *Zostera*, with peak densities during summer months and minimum densities during winter months (Beckley, 1983; Pollard, 1984; Whitfield, 2016). The arrival of juveniles of most marine migrants into estuaries during spring and summer thus also coincides with maximal seagrass development (Beckley, 1983; Bennett, 1989; Pollard, 1984; Whitfield, 2016). This cyclical increase and decrease in fish abundances over the seasons could also explain the fish abundance results documented in the current study. As the water temperatures and *Z. capensis* densities increased, there was a visible increase in fish abundances of both sparid and mugilid species at the Knysna sampling sites.

The Knysna sites during the current study, were not permanently inundated and were either partially or completely exposed over every low tide. Tides, water depth, habitat complexity and structure are key drivers in influencing the selection of nursery areas by fish, with juveniles attracted to permanently inundated littoral habitats that are structurally complex (Pollard, 1984; Connolly and Hindell, 2006; Becker *et al.*, 2012;). Whitfield (2016) showed in his review that fish assemblages in seagrass beds that were covered by deeper water were more abundant and diverse than the fish assemblages in seagrass beds that were covered by a shallow water depth. The abundance and diversity of food resources also vary according to the amount of time the habitat is submerged, with areas that are permanently submerged having a higher biomass of potential micro-algal resources compared to those that are completely exposed on each low tide (Becker *et al.*, 2012; Whitfield, 2016). This factor could thus have influenced the abundance as well as the diversity of fish species observed during the current study.

5.2. Underwater visual monitoring versus seine netting

To put the method of underwater visual monitoring to the test of recording fish abundance and diversity in an estuarine environment, seine net sampling was conducted on the same days as video recording to compare the two methods during upwelling and non-upwelling conditions. Spearman's rank correlation analysis showed that there was indeed a positive linear correlation between the two sampling methods. Although not all the fish observed in the video footage were always caught in the net and *vice versa* on every sampling occasion, there was a similar abundance pattern of species recorded using both sampling methods.

The video analysis showed that during upwelling conditions (12, 13, 26 and 27 November 2014), when water temperatures were between 11°C and 16°C, the number of sparid species seen was very low. The most observed fishes during cool water conditions were mugilids (probably *Liza richardsonii* and *L. dumerili*), as well as the evil-eyed puffer fish (*Amblyrhynchotes honckenii).* As the water temperature increased to between 21°C and 23°C (28 and 29 January 2015, 10 and 11 February 2015), there was a substantial increase in sparids such as *Sarpa salpa* and *Diplodus capensis,* and to a lesser extent *R. globiceps*. Mugilidae were still recorded on the camera footage but comprised a much smaller percentage of the observed fish.

The seine netting analysis showed a similar trend to the camera footage. During the cool water temperature sampling, the fish captured in the net consisted of mostly *L. richardsonii, L. dumerili,* and *A. honckenii*. As soon as the water temperature increased, large numbers of sparids were captured including *S. salpa, D. capensis* and *R. globiceps*. These results showed the effect that water temperature has on the presence of fish as well as their distribution in estuarine environments.

During cool upwelling events many marine fish inhabiting the Eastern and Western Cape coasts of South Africa take refuge in estuarine systems where the water temperatures are higher (Whitfield, 1998). Therefore fish species in the Knysna Estuary that are sensitive to cooler water may move higher up the estuary towards the middle and upper reaches where warmer water temperatures prevail during upwelling events. This would account for the low abundances and diversity of fish species during video recordings and seine netting during an upwelling event in the currents study.

Water temperature is an important factor influencing fish abundance in estuaries (Whitfield, 1994). Bennett (1989) found that there are clear seasonal changes in fish abundance in estuaries of the southern Cape. During the winter months when freshwater input is high and the water temperatures are low, fish species abundance is low, while during the summer months when freshwater input declines and water temperatures increase, fish species abundance increases. Pollard (1984) also noted that coastal fish diversity and abundance in the north-western Atlantic region increased during spring and summer months as *Zostera* biomass and water temperatures increased, with a reverse pattern recorded in autumn and winter as fish diversity and abundance declined in conjunction with decreasing water temperatures and *Zostera* biomass. Although the recruitment of most juvenile fishes occur during summer months when water temperatures are higher (Whitfield, 1994), in this study it was shown that during upwelling events in summer months, the number of fish can undergo major declines in association with the cooler upwelled waters.

Rhabdosargus holubi has a peak recruitment period into southern Cape estuaries from August to April, with high numbers of this species present in summer and a decline of postlarval and early juvenile abundance during the winter months (Bennett, 1989; Whitfield and Kok, 1992; Whitfield, 1998). This species is present within the Knysna Estuary year round with high abundances in the middle and upper reaches of the estuary and is closely associated with *Zostera capensis* habitats (Whitfield and Kok, 1992). The same pattern was found in the current study with increased abundances of *R. holubi* during the summer months associated with *Z. capensis* beds and a decrease in abundance during the winter months.

Lithognathus lithognathus has a peak recruitment period into the Knysna estuary during November but enters estuaries in the southern Cape from September to January (Bennett, 1989; Whitfield and Kok, 1992). This species is most abundant in the middle and upper reaches of the Knysna Estuary (Whitfield and Kok, 1992) which may account for its low abundance in the lower reaches during the current study where it was more associated with bare substrata than *Z. capensis* habitats. *Diplodus capensis* is known to enter the Knysna Estuary during spring and summer but has a peak recruitment period between October and December (Whitfield and Kok, 1992). This species is most abundant in the lower and middle reaches of the estuary (Whitfield and Kok, 1992). *Sarpa salpa* has a peak recruitment period into the Knysna Estuary between September and December and is closely associated with *Z. capensis* habitats in the lower and middle reaches of the estuary (Whitfield and Kok, 1992). The substantial increase in *D. sargus* and *S. salpa* numbers during spring and summer sampling might be explained by their recruitment period into the estuary as well as their preference for the lower and middle reaches of the estuary.

Lithognathus mormyrus recruits into the Knysna Estuary from September to April and is generally confined to the lower and middle reaches of the estuary (Whitfield and Kok, 1992). *Rhabdosargus globiceps* has a similar recruitment period into the Knysna Estuary between September and December, with a peak in November and December and occurring in the lower half of the estuary (Whitfield and Kok, 1992). *Diplodus hottentotus* also has a recruitment period into the Knysna Estuary from September to December and is also limited to the lower half of the estuary (Whitfield and Kok, 1992). These three sparid species had low abundances during the 2014 and 2015 sampling period but were more abundant in the historical data set.

Liza richardsonii recruits into the Knysna Estuary and other southern Cape estuaries throughout the year, with a peak between November and May (Bennett, 1989; Whitfield and Kok, 1992). This species is most abundant in the lower and middle reaches of the estuary (Whitfield and Kok, 1992). *Liza tricuspidens* has a more reduced recruitment period into the Knysna Estuary from October to May, with a peak from October to December (Whitfield and Kok, 1992; Whitfield, 1998). This species is most abundant in the lower half of the estuary (Whitfield and Kok, 1992). *Liza dumerili* has a peak recruitment period into the Knysna Estuary between November and June, although the juveniles of this species can enter the estuary at any time of the year (Whitfield and Kok, 1992). This species is most abundant in the lower and middle reaches of the estuary (Whitfield and Kok, 1992). The high abundance of *L. richardsonii* and *L. dumerili* during the current study can thus be explained by their year round recruitment as well as their preference for the extensive habitats available in the lower half of the estuary.

Mugil cephalus recruits into southern Cape estuaries from August to December with peak immigration in the Knysna Estuary occurring between September and November (Bennett, 1989; Whitfield and Kok, 1992). This species is abundant throughout the estuary (Whitfield and Kok, 1992). *Myxus capensis* recruits into southern Cape estuaries throughout the year, with a main recruitment period into the Knysna Estuary between July and November and a peak during October and November (Bennett, 1989; Whitfield and Kok, 1992). This species has been recorded to leave the estuary at certain times when they utilize the rivers connected to the estuary (Whitfield and Kok, 1992). These two mugilid species were recorded in very low numbers during 2014 and 2015 and the same was seen in the historical dataset.

The use of remote underwater video cameras has been shown to be very useful in studying fish assemblages that are associated with specific habitat patches (Becker *et al*., 2010) such as those sampled in the current study. A study conducted by Cappo *et al*. (2004) compared the use of baited remote underwater video stations (BRUVS) with prawn trawls to assess fish biodiversity in a reef marine park and they determined that both techniques detected the same temporal and spatial patterns in fish biodiversity. Similarly, Gladstone *et al*. (2012) found that the differences in fish species structure and abundance between unvegetated sand and seagrass habitats recorded by BRUVS were supported by other studies that used trawls and seine nets as sampling methods in temperate estuaries. The above findings supported the approach adopted for the current study and the two sampling methods in the Knysna Estuary produced similar fish diversity results.

A big advantage of using remote underwater video cameras is the non-destructive nature of this sampling method (Cappo *et al*., 2004; Becker *et al*., 2010; Lowry *et al*., 2011; Ellender *et al*., 2012; Gladstone *et al*., 2012; Weyl *et al*., 2013), which gives the option of observing the behaviour of fish under natural conditions and thus being able to connect the fish to that habitat more directly as well as examining fish assemblages across various habitats without using destructive sampling (Cappo *et al*., 2004; Becker *et al*., 2010; Becker *et al*., 2012). This provided insight into the behaviour of fish observed in this specific study. These non-destructive methods are essential in marine protected areas (Lowry *et al*., 2011; Pelletier *et al*., 2011; Gladstone *et al*., 2012) such as the Knysna Estuary which is part of the Garden Route National Park.

There are, however, negative aspects to using underwater cameras as certain fish species are not recorded (Becker *et al*., 2012). Work by Becker *et al*. (2010) in the East Kleinemonde Estuary in the Eastern Cape of South Africa showed that benthic fish species such as gobies and soles were not recorded in the footage although they were caught in seine nets at the same sites. In the current study, the same species that were observed in the video footage were caught in the seine net, although not always in the same proportional abundance.

Another problem regarding video camera analyses is the use of the MaxN value to calculate fish abundance which can sometimes be misleading, often overestimating the numbers of schooling species and underestimating the numbers of loose aggregations of fishes (Cappo *et al.*, 2004; Becker *et al.* 2010; Pelletier *et al.*, 2011). An example from Becker *et al.* (2010) is the low MaxN recorded for abundant species such as *R. holubi,* where not more than two individuals were observed at a time, while shoaling species such as *Gilchristella aestuaria* or *Atherina breviceps* were recorded to have high MaxN values. This might have happened in the current study as well with regard to the numbers of fish observed in video footage as the mugilid species were most often recorded in big schools and resulted in high MaxN values while species such as *R. holubi* and *D. hottentotus* were mostly two individuals at a time and thus resulted in low MaxN values. Processing time of video footage can also be a disadvantage with this technique as extra time is needed to go over the footage and identify fishes (Pelletier *et al.*, 2011), especially when significant amounts of footage are collected.

Another problem with remote underwater video cameras is the difficulty in distinguishing similar looking fish species from each other (Becker *et al.*, 2010; Pelletier *et al.*, 2011; Becker *et al.*, 2012). This applied to the current study when it came to distinguishing the different mugilid species from one another. These fish, therefore, could only be identified to family level. It should be noted, however, that an advantage of working with video footage is that it can be watched repeatedly by several observers to identify any fish that are not clear to the original observer, and guides can also be used to identify fish (Pelletier *et al.*, 2011). Water turbidity also plays a major role in being able to identify closely related fish species, with increasing turbidity making the fish less distinguishable on the video footage (Gladstone *et al.*, 2012). This problem was also noted by Cappo *et al.* (2004) and was the reason why the recordings from some sampling days had to be discarded from the final data set used in the current study.

5.3. Historical data

The historical fish data set showed that there was a difference in fish species composition and fish abundance across the different habitats (bare substratum, sparse *Z. capensis* and dense *Z. capensis*), the different regimes (bay, lagoon and estuary) as well as across the four seasons. Unfortunately there was no clear separation of the fish groupings using these variables when analysing the data using MDS plots.

The species that contributed most to the within group similarities were the sparids *R. holubi*, *D. sargus* and *L. lithognathus*, and the mugilid *L. richardsonii*. The two species most dominant at bare substratum habitats were *R. holubi* and *L. lithognathus*, in sparse *Z. capensis* it was *R. holubi* and *L. richardsonii*, and in dense *Z. capensis* it was *R. holubi* and *D. sargus*. For spatial patterns, *D. sargus* and *R. holubi* were most dominant in the bay regime, *R. holubi*, *L. richardsonii* and *L. lithognathus* in the lagoon regime, and *L. lithognathus* and *R. holubi* in the estuary regime. In terms of seasonality, *R. holubi* and *D. sargus* were most dominant during spring, in summer it was *R. holubi* and *L. richardsonii*, in autumn it was *R. holubi* and *D. sargus*, and in winter *L. richardsonii* and *R. holubi*. From the above it can be concluded that *R. holubi* was one of the most dominant species, being present in all habitats, all regimes and all seasons.

Wallace *et al.* (1984) identified 81 fish species in South African estuaries that are exclusively or partially dependent on estuaries for their survival. Of these there are 22 species that rely completely on estuaries as nursery grounds during their juvenile stages which include *L. lithognathus, M. cephalus, M. capensis* and *R. holubi* (Wallace *et al.*, 1984). Then there are also species that use both estuaries and the sea as nursery grounds, including *L. dumerili, L. tricuspidens, L. richardsonii, D. sargus, R. globiceps* and *S. salpa* (Wallace *et al.*, 1984). Although juvenile fish recruit into estuaries mainly during spring and summer months, adult fish abundance also increases seasonally as certain species enter estuaries to feed (Wallace *et al.*, 1984). As documented in the current study, most of the species mentioned above were observed in the Knysna Estuary using both sampling methods (underwater video monitoring and seine netting) as well as in the historical data set. The seasonal increase of juvenile and subadult fish in the estuary was also very clear and can be related to the increase in *Z. capensis* habitat and associated food sources during summer (Pollard, 1984).

Whitfield and Kok (1992) state that the majority of fish species that are found in South African estuarine systems are of marine origin and that these systems are most often used as nursery sites for marine migrants which spawn at sea (Ter Morshuizen and Whitfield, 1994). Fishes such as *R. globiceps* and *L. richardsonii* use the favourable nursery aspect of estuaries opportunistically while species such as *R. holubi* and *L. lithognathus* are completely dependent on estuaries as nursery grounds (Whitfield and Kok, 1992). *Rhabdosargus holubi, L. lithognathus, D. sargus, S. salpa, L. richardsonii, L. tricuspidens, L. dumerili, M. cephalus* and *M. capensis* are the most dominant species in the Knysna Estuary, with other species such as *Spondyliosoma emarginatum*, *L. mormyrus*, *R. globiceps* and *D. hottentotus* also being well represented (Whitfield and Kok, 1992). The same species were found to be dominant in the underwater video monitoring and the seine net sampling conducted in 2014 and 2015 showing that the Knysna Estuary is still a valuable nursery and foraging area for these fish.

Whitfield *et al.* (1989) documented 97 species from *Z. capensis* habitats in estuaries throughout South Africa. Of these, *L. dumerili, L. richardsonii* and *R. holubi* were found to be the most abundant fish species associated with this habitat (Whitfield *et al*., 1989) as was the case during the current study. A study conducted by Beckley (1983) on the fishes associated with *Z. capensis* in the Swartkops Estuary of South Africa showed that very similar species were found in this habitat compared to the current study. Six mugilid species were documented, the most abundant being *M. cephalus, L. dumerili, L. richardsonii* and *L. tricuspidens* (Beckley, 1983). *Rhabdosargus holubi* was one of the most dominant species in *Z. capensis* bed catches, with a high abundance of juveniles in spring and summer. Similarly *D. sargus*, *R. globiceps* and *D. hottentotus* juveniles also showing an influx to *Z. capensis* habitats during spring and summer months (Beckley, 1983). The same pattern was observed in the current study, with a spring and summer influx of these sparid species. Other sparids associated with *Z. capensis* beds were also documented in the Swartkops Estuary, although in lower abundances, and included *S. emarginatum* and *S. salpa*. (Beckley, 1983) which were recorded to be associated with *Z. capensis* beds in the Knysna study. Beckley (1983) also found that *L. lithognathus* was not associated with Z. capensis beds in the Swartkops Estuary, a pattern that was also reflected in the current study where this species mainly observed in the vicinity of bare substratum habitats.

A study conducted on the fishes associated with *Z. capensis* in the Kariega Estuary showed that *R. holubi* was one of the most dominant species caught within this habitat (Ter Morshuizen and Whitfield, 1994). Other species that were also caught in these *Z. capensis* beds included several mugilid species, *D. capensis*, *D. hottentotus, L. lithognathus,* and *S. emarginatum* (Ter Morshuizen and Whitfield, 1994). *Rhabdosargus holubi* was most abundant in the middle and upper reaches of the estuary and the mugilid species were most abundant in the lower reaches (Ter Morshuizen and Whitfield, 1994). The evil-eye puffer fish, *A. honckenii,* was associated with *Z. capensis* beds in the Kariega Estuary (Ter Morshuizen and Whitfield, 1994) a pattern that was also recorded in the current study at the Knysna Estuary.

Hanekom and Baird (1984; 1988) found that *R. holubi* was the dominant fish species associated with *Z. capensis* beds in the Kromme Estuary of South Africa. The mugilid *L. dumerili* was caught in high abundances in non-*Zostera* areas in this estuary while others such as *L. richardsonii* and *L. tricuspidens* were caught in quite low numbers in both habitats (Hanekom and Baird, 1984). In contrast, *M. cephalus* was caught mostly in *Zostera* areas (Hanekom and Baird, 1984). A study on the Swartvlei Estuary showed that *D. sargus* and *S. salpa* juveniles were only caught in or around *Z. capensis* beds (Whitfield, 1988). While the majority of *L. richardsonii* juveniles were caught over unstructured habitats, 48% were caught in *Z. capensis* beds and 98% of *R. holubi* were also caught in the latter habitat (Whitfield, 1988). The findings of the current study supports that which was found by Whitfield (1988) with sparid species being strongly associated with *Z. capensis* habitats and mugilids more abundant at or near bare substratum habitats.

In a review of the fish communities associated with seagrasses, Pollard (1984) found that in the Mediterranean (where *Z. marina* is present) the greatest abundance and diversity of juvenile fish occurred during summer and autumn. Most fish species in the north-western Atlantic area preferred the naturally vegetated areas of *Z. marina* (and some algae) to unvegetated substrata. Both the diversity and density of fishes were higher in these vegetated areas compared to unvegetated areas (Pollard, 1984). Studies done on *Zostera* in the north-eastern Pacific showed that juveniles are commonly found in these *Zostera* beds and can thus be regarded as important nursey areas, although adult fish of some species are also often recorded in these habitats (Pollard, 1984).

Japanese *Zostera* in the north-western Pacific is noted for its importance as nursey areas for commercially important fish species and higher species richness and density was observed in *Zostera* beds compared to unvegetated areas (Pollard, 1984). The *Zostera* beds in the south-western Pacific showed the same trend, with recruitment of economically important juvenile fishes to this habitat occurring during the spring and summer months (Pollard, 1984). Sparid species are commonly associated with *Zostera* habitats and are one of the top “ten most important seagrass-fish families” in different seagrass types over a wide array of geographical localities (Pollard, 1984). Sparid species were also strongly associated with *Z. capensis* habitats in the Knysna Estuary and much more frequently linked to these habitats than bare substratum areas.

In a review by Heck *et al.* (2003), 46% of the studies examined showed that there was a greater abundance of fishes in seagrass beds, 26% showed a greater abundance of fish in other habitats, and 28% showed no difference in fish abundance between seagrass and other habitats. The authors attributed this result to the fact that the other habitats were also structurally complex, such as mangroves and salt marshes, which also support high densities of fish and are known to play a nursery role for many juvenile fish species (Heck *et al*., 2003). Similarly a review by Whitfield (2016) showed that, when compared to other habitats such as mangrove, salt marsh and unvegetated areas, seagrass beds generally supported higher species diversity and greater fish abundance than any of the other habitats.

There is also a hypothesis that geography may play a major role in the relative importance of seagrass beds as fish nursery areas. Heck *et al*. (2003) has shown that these habitats played a more important role in the northern hemisphere than the southern hemisphere. The above authors cautioned that this may change as more studies on seagrasses are completed in the southern hemisphere. Bell *et al*. (1988) showed that the location of a seagrass bed within an estuary could also play a significant role in the abundance of juvenile fish within that habitat. A similar conclusion was documented in the Kariega Estuary where Ter Morshuizen and Whitfield (1994) suggested that factors such as availability of specific food items, distance of the *Z. capensis* bed from the sea and abundance of predators may all play a part in the determining of fish species distributions within this system.

According to reviews by Heck *et al*. (2003) and Whitfield (2016), seagrass meadows had a significant positive influence on the survival of juvenile fishes when compared to other habitats. Juvenile survival in seagrass beds were seen to be significantly higher than in unstructured areas such as sand and soft sediment habitats (Heck *et al*., 2003; Whitfield, 2016). When seagrass beds were compared to other structural habitats such as macroalgal beds, there was no difference in the survival of juvenile fishes (Heck *et al*., 2003). Growth rates of juveniles were documented to be much greater in seagrass meadows when compared to bare substratum habitats (Heck *et al*., 2003; Whitfield, 2016).

The high abundance of fish in the Knysna Estuary has been partially attributed by Whitfield and Kok (1992) to the strong marine influence associated with the deep, permanently open mouth, which also makes it a more stable and predictable nursery area compared to nearby temporarily open/closed estuaries (Whitfield *et al.*, 1989). Fish migration patterns have been shown to be directly linked to the seasonal variations in species abundance, composition and size distribution of marine fishes in estuaries (Bennett, 1989; Whitfield and Kok, 1992). In southern Cape estuaries the peak recruitment of fishes into estuaries occurs during the summer months when food source availability is at its maximum (Bennett, 1989; Whitfield and Kok, 1992).

5.4. Fish behaviour

The GLMs run on the three different fish behaviours (feeding, rapid swimming and slow meandering) showed that there were no significant differences in the behaviour shown by the two different fish families between habitats or seasons. Feeding behaviour appeared to occur more during the spring and summer months for both families in both vegetated and unvegetated habitats. The occurrence of rapid swimming was higher in summer months for Sparidae, and spring months for Mugilidae, with both families having higher occurrences of rapid swimming behaviour over bare substratum areas than *Z. capensis* areas. Slow meandering behaviour was seen to be more prevalent in the Mugilidae than the Sparidae with both families exhibiting this behaviour more during spring and summer months.

The high prevalence of all three of the behaviours during spring and summer could be due to the fact that there were more fish observed during these seasons and thus more incidences of different behaviours could be observed. The increased activity recorded in the spring and summer months may be linked to the effect of warmer water temperatures on poikilotherms, such as fishes (Bullock, 1955; Coutant, 1976). Increasing water temperatures are likely to directly impact on the feeding activity, metabolic and digestive processes and reproductive development in fishes (Coutant, 1976), thus explaining the increase in activity observed from the video footage during these seasons.

Fish behaviour can be influenced by plant morphology as well as plant distribution and associated food sources, e.g. it has been shown that aquatic plant beds provide both refuge for juvenile fish as well as a food source (Dibble *et al*., 1996). Since the leaves and stems of aquatic plants provide a surface for attachment, macroinvertebrate diversity and abundance are higher in these habitats compared to unvegetated areas and thus provide more prey for fishes (Dibble *et al*., 1996; Whitfield, 2016). The structural complexity of aquatic plants such as *Zostera* may reduce the risk of predation on juvenile fish and they create swimming and visual barriers for larger predatory fish (Dibble *et al*., 1996). It has also been shown that foraging efficiency of predatory fish declines as the habitat becomes more spatially complex (Pollard, 1984; Dibble *et al*., 1996). This could explain the higher frequency of rapid swimming behaviour in sparid species over bare substratum habitats as there is no protection from large predatory fishes, as well as the slow meandering behaviour over *Z. capensis* habitats where refuge is readily available for fishes.

In the East Kleinemonde Estuary of South Africa, Becker *et al.* (2010) found that over bare areas fish frequently displayed rapid swimming behaviour, while in vegetated areas slow meandering was the dominant behaviour type. These authors linked the above behaviour to differences in predation risk and/or food availability in the different habitats. Both Becker *et al.* (2010) and Leslie (2015) recorded *R. holubi* exhibiting rapid swimming behaviour mostly over bare sand habitats and slow meandering in structured habitats and Leslie (2015) attributed this to the complexity of the *Z. capensis* habitat that provides protection from predators. The same behavioural traits were seen in *R. holubi* in the current study with this species displaying a higher proportion of rapid swimming over bare substratum habitats and slow meandering in *Z. capensis* habitats. Becker *et al.* (2012) also found that fish behaviour was strongly related to habitat structure. The Mugilidae showed feeding behaviour along the edges of seagrass beds in the Bushmans Estuary, often feeding directly off the seagrass blades as well as the sand next to the *Z. capensis* (Becker *et al.*, 2012). The same behaviour was documented during the current study, with mugilids sometimes foraging directly from the surface of *Z. capensis* blades although most mugilids in the Knysna Estuary seemed to be present and feed in bare substratum areas.

5.5. *Ulva* versus no *Ulva*

The occurrence of an *Ulva* bloom during the second half of the sampling period in 2015 provided an opportunity to assess the influence of a macroalgal bloom on the relative abundance and behaviour of fish in *Z. capensis* and bare substratum habitats. This data set was restricted to August 2014 and August 2015 to eliminate any bias associated with season and water temperature.

A GLM run on the data showed that there was a difference in the abundance of fish between the two years of sampling. As shown above, the abundance of the Sparidae decreases during the winter months, which explains why there were less Sparidae observed during this season. However, it should be noted that the small number of Sparidae recorded during the bloom winter season was even lower than the non-bloom winter period. Differences were even more substantial when comparing the abundances of Mugilidae in the *Z. capensis* habitat compared to the degraded *Z. capensis* (following invasion by *Ulva*). As the *Z. capensis* habitat was much more affected by the macroalgal bloom than the bare substratum habitat, it is suggested that the *Ulva* impacted negatively on the abundance of Mugilidae observed in this particular habitat.

Whitfield and Pattrick (2015) reported that the smothering of *Zostera* beds by algal blooms can decrease the value of these specific habitats to juvenile fish and lead to substantial changes in the fish assemblage structure. The presence of epiphytic filamentous algae can reduce the suitability of *Zostera* beds as nursey habitats for fish (Whitfield and Pattrick, 2015). These findings could explain the results seen in the current study. As most of the *Z. capensis* beds were smothered by macroalgae in the winter of 2015, the protection and food usually provided by this habitat was no longer available. Secondly, the smothering of the bare substratum habitats by *Ulva* would have reduced the main feeding ground used by mugilids, which would have had a negative feedback for the densities of these species.

In a study conducted on the Little Egg Harbour and Great Bay estuarine systems in southern New Jersey (USA), Sogard and Able (1991) found that when a comparison between *Zostera marina* habitat and macroalgal habitat (*Ulva lactuca*) was undertaken, the former had higher densities of fish. Sogard and Able (1991) concluded that the macroalgae did not provide an equivalent habitat substitute for *Zostera* for the associated fish species. Wherever *Zostera* beds are converted into *Ulva* beds due to eutrophication, a decline in both the species diversity and abundance of juvenile fishes can be expected (Sogard and Able, 1991; Whitfield, 2016). In addition, macroalgal mats can have adverse effects on the resident invertebrate organisms and fishes of that ecosystem by lowering dissolved oxygen levels in both the water and sediment (Sogard and Able, 1991).

An interesting point to make here is that loose leaves of *Ulva* often obstructed the video camera by covering the lens completely and thus producing no results as nothing could be seen in the water column. Becker *et al.* (2012) found the same problem when recording in seagrass beds and decided to restrict recording to the edge of the beds. Unfortunately this was not an option during this study as the *Ulva* leaves often obstructed the cameras that were placed in bare substratum habitats. Although the *Ulva* bloom in the estuary had an impact on the *Z. capensis* beds, it must be remembered that these plants also undergo natural senescence during winter months as part of their cyclical growth rates and this similarly causes a decrease in fish abundance (Whitfield, 1984; Dibble *et al.*, 1996; Whitfield, 2016).

5.6 Conclusion and future work

Zostera capensis meadows occupy a large area, approximately 421 ha, within the Knysna Estuary (Barnes, 2010b) but little is known about their importance to associated fish assemblages. With *Z. capensis* meadows being under increased pressure from anthropogenic influences, it is important to establish which fish species and families utilize these habitats and what role do they play in the ecology of fish assemblages within the Knysna Estuary. The primary objective of this study was to compare the use of *Z. capensis* and adjacent bare sediment areas by mainly large juvenile and subadult sparids and mugilids using different techniques.

There were two key questions;

1. Was a different fish family dominant in *Z. capensis* meadows when compared to the adjacent areas without vegetation?
2. What is the relevant density and behaviour of fishes within *Z. capensis* meadows and adjacent unvegetated areas?

The two main hypotheses were as follows;

1. Mugilidae are likely to be more dominant in the unvegetated areas of the estuary littoral and Sparidae are likely to predominate within the *Z. capensis* bed areas of the estuary littoral.
2. The non-destructive underwater video monitoring method would yield similar fish composition data to seine netting sampling of the identical sites.

Both hypotheses were assessed using data collected during this study and the analysis of historical unpublished data. Overall, Mugilidae were more abundant at bare unvegetated areas where they did most of their foraging. Sparidae were more abundant in the *Z. capensis* beds, which was also the habitat where they primarily foraged. With regards to the comparison of two different sampling methods, namely underwater video monitoring and seine netting, similar patterns arose with regards to the fish species observed in camera footage and those captured in the seine net, although the abundances were not always comparable.

Unfortunately all sampling methods used for fish monitoring are selective to some degree, whether with respect to morphological type or life stage, fish size or species, habitat type or place in the water column (Franco *et al.*, 2011). Since BRUVS, remote underwater video cameras, underwater visual censuses, trawls and nets are very different in their method of sampling, each one will record a different subgroup of fish from the same assemblage (Gladstone *et al.*, 2012). Thus a combination of multiple sampling methods would seem to be the best way to gain the most complete information set on fish species assemblages in any one area (Harvey *et al.*, 2007; Gladstone *et al.*, 2012; Lowry *et al.*, 2011).

With regards to future studies, Pollard (1984) found that fish were more abundant at night. Similarly, Whitfield (2016) notes that, in several studies, night-time catches of fish in seagrass were higher than those in day-time catches. Therefore, it would be valuable to document whether this trend is also true for video recording and whether the behaviour of the fish species changes at night, e.g. fish might be using vegetated areas for sleeping. This could be achieved by the use of a DIDSON (Dual-frequency identification sonar). An increase in video recording time would also be beneficial to observe fish over a longer time period than was possible in this study and thereby account for known variables such as tidal and diel effects (Lowry *et al.*, 2011).

A major shortfall of the current study was the use of only one area in the estuary for sampling. Future work should include multiple cameras placed in different sections of the estuary to compare fish composition, abundance and behaviour in all three regimes. This would give a more complete assessment of the ichthyofauna in terms of different families and species utilizing different regimes of the estuary, as well as ascertaining whether certain fish change their preferred habitat from *Z. capensis* to other submerged macrophytes in different parts of the estuary. It could also give an indication as to how different fish species react to salinity gradients especially during flooding events. The disappearance of sparid species from the lower reaches during marine upwelling events may also be answered by the simultaneous deployment of cameras in all three reaches of the estuary to see whether sparids move up the estuary when these events occur.

Climate change is altering many environments, and seagrass meadows are not immune to this type of change (Roessig *et al.*, 2004). Major changes in the distribution and extent of seagrass meadows are expected and this will inevitably have immense impacts on fish assemblages within estuaries (Roessig *et al.*, 2004; Whitfield *et al.*, 1989; Whitfield, 2016). Along with this there are also strong indications that estuarine macrophytes globally are under threat from a diverse array of anthropogenic stresses (Orth *et al.*, 2006; Adams, 2016) and currently 15% of seagrass taxa are considered as threatened (Hughes *et al.*, 2008).

Evidence that the destruction or degradation of littoral macrophyte habitats leads to the decline or complete loss of important nursey areas are in abundance (Pollard, 1984; Whitfield *et al.*, 1989; Roessig *et al.*, 2004; Orth *et al.*, 2006). Although some of the fish species associated with these habitats might be able to survive without them, it is very likely that growth rates will be lower and mortality rates higher under such a scenario. Unfortunately those species that are completely dependent on these habitats will be faced with the major threat of localised extinction where such habitats are lost (Roessig *et al.*, 2004; Whitfield, 2016).

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Referencing formatted according to African Journal of Marine Science

Appendices

Appendix 1

Ichthyofaunal families and species associated with *Zostera capensis* beds in nine estuaries and one lagoon in South Africa. Asterisk (*) = presence of species.

Family	Species	Richards Bay	Mngazana Estuary	Kariega Estuary	Bushmans Estuary	Sundays Estuary	Swartkops Estuary	Kromme Estuary	Knysna Estuary	Swartvlei Estuary	Langebaan Lagoon
Ambassidae	*Ambassis gymnocephalus*	*	*								
	Ambassis natalensis	*	*								
Atherinidae	*Atherina breviceps*		*	*	*	*	*	*	*	*	*
Bagridae	*Galeichthys feliceps*			*				*			
Blenniidae	*Istiblennius dussumieri*								*		
	Omobranchus spp.		*								
	Omobranchus woodi						*				
	Parablennius cornutus					*					
Bothidae	*Bothus pantherinus*		*								
Carangidae	*Caranx ignobilis*	*									
	Caranx sexfasciatus	*							*		
	Lichia amia						*	*	*		
	Trachurus trachurus						*		*	*	
Chaetodontidae	*Chaetodon lunula*		*								
	Chaetodon marleyi			*					*		
Clinidae	*Clinus agilis*								*		
	Clinus superciliosus			*	*		*	*	*	*	*
Clupeidae	*Gilchristella aestuaria*		*	*	*	*	*	*	*	*	
	Hilsa kelee	*									
	Sardinops sagax								*		
Dasyatidae	*Dasyatis pastinaca*								*		
Diodontidae	*Lophodiodon calori*						*				
Dussumieriidae	*Estrumeus whiteheadi*								*		
Eleotridae	*Eleotris fusca*		*								
Elopidae	*Elops machnata*				*						
Engraulidae	*Engraulis capensis*								*		
	Stolephorus holodon						*				
Ephippidae	*Platax pinnatus*		*								
Fistulariidae	*Fistularia petimba*		*						*		
Gerreidae	*Gerres acinaces*	*	*								
	Gerres filamentosus	*	*								
	Gerres oyena	*									

Family	**Species**	**Richards Bay**	**Mngazana Estuary**	**Kariega Estuary**
Gobiidae	*Caffrogobius multifasciatus*		*	*
	Caffrogobius natalensis			*
	Caffrogobius nudiceps		*	*
	Glossogobius callidus			*
	Gobiopsis pinto			*
	Oligolepis acutipennis		*	
	Psammogobius knysnaensis	*	*	*
	Redigobius dewaali			*
Gobiesocidae	*Apletodon pellegrini*			
Haemulidae	*Plectorhinchus gibbosus*	*		
	Pomadasys commersonnii	*		*
	Pomadasys kaakan	*		
	Pomadasys olivaceum			
Hemiramphidae	*Hemiramphus far*	*		
	Hyporhamphus capensis			*
Kuhliidae	*Kuhlia mugil*		*	
Leiognathidae	*Leiognathus equula*	*		
	Secutor insidiator	*		
Lutjanidae	*Lutjanus argentimaculatus*			
	Lutjanus fulviflamma	*	*	
Monacanthidae	*Stephanolepis auratus*			
Monodactylidae	*Monodactylus argenteus*	*	*	
	Monodactylus falciformis			*
Mugilidae	*Liza dumerili*	*	*	*
	Liza macrolepis	*	*	
	Liza richardsonii			
	Liza tricuspidens			
	Mugil cephalus	*		*
	Myxus capensis			
	Valamugil cunnesius	*		
	Valamugil buchanani	*		
	Mugilidae			*
Mullidae	*Parupeneus rubescens*			
Paralichthyidae	*Pseudorhombus arsius*	*		
Platycephalidae	*Platycephalus indicus*	*		*
Pomatomidae	*Pomatomus saltatrix*	*		*
Sciaenidae	*Argyrosomus japonicus*			*
	Johnius dussumieri	*		

Bushmans Estuary	Sundays Estuary	Swartkops Estuary	Kromme Estuary	Knysna Estuary	Swartvlei Estuary	Langebaan Lagoon
*	*	*	*	*	*	
				*	*	
				*	*	*
*	*	*	*	*	*	*
				*		
	*	*	*	*		
	*	*	*	*		
				*	*	
*				*		
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		*				
*		*				
*	*	*	*	*	*	
*	*	*	*	*	*	
				*		
*	*	*	*	*	*	*
*	*	*	*	*	*	
*	*	*	*	*	*	
		*	*	*	*	
	*			*		
		*				
				*		

Family	**Species**	**Richards Bay**	**Mngazana Estuary**	**Kariega Estuary**	**Bushmans Estuary**	**Sundays Estuary**	**Swartkops Estuary**	**Kromme Estuary**	**Knysna Estuary**	**Swartvlei Estuary**	**Langebaan Lagoon**
Scorpaenidae	*Parascorpaena mossambica*		*								
	Pterois miles		*		*						
Serranidae	*Epinephelus andersoni*	*							*		
	Epinephelus guaza						*				
Siganidae	*Siganus sutor*	*			*						
Sillaginidae	*Sillago sihama*	*									
Soleidae	*Heteromycteris capensis*			*	*	*	*	*	*	*	*
	Solea bleekeri	*	*		*	*	*	*	*	*	*
Sparidae	*Acanthopagrus berda*	*	*								
	Diplodus hottentotus		*	*	*	*	*		*		
	Diplodus capensis	*	*	*	*	*	*	*	*	*	*
	Lithognathus lithognathus			*	*	*			*	*	
	Lithognathus mormyrus								*		
	Pagellus natalensis									*	
	Rhabdosargus globiseps			*			*		*		*
	Rhabdosargus holubi	*	*	*	*	*	*	*	*	*	
	Rhabdosargus sarba	*	*							*	
	Sarpa salpa		*			*	*		*	*	*
	Spondyliosoma emarginatum			*	*	*	*		*		
	Sporodon durbanensis						*			*	
Sphyraenidae	*Sphyraena* spp.	*							*		
Syngnathidae	*Hippocampus capensis*								*		
	Syngnathus temminckii			*	*	*	*	*	*	*	*
Synodontidae	*Saurida undosquamis*		*								
Terapontidae	*Amblyrhynchotes honckenii*						*		*		
	Arothron hispidus		*		*		*				
	Arothron immaculatus	*	*				*		*		
	Chelonodon laticeps	*									
	Pelates quadrilineatus	*									
	Terapon jarbua	*	*						*		
Torpedinidae	*Torpedo fuscomaculata*			*					*		

(Beckley, 1983; Beckley, 1984; Hanekom and Baird, 1984; Ter Morshuizen and Whitfield, 1994; Whitfield, 1988; Whitfield *et al*., 1989)

Appendix 2

List of species observed during video recordings and caught during seine net sampling.
Asterisk (*) = presence of species

Family	Species name	Common name	Video footage	Seine net
Atherinidae	*Atherina breviceps*	Cape silverside	*	*
Carangidae	*Lichia amia*	Garrick	*	*
Clupeidae	*Gilchristella aestuaria*	Gilchrist's round herring	*	*
Dasyatidae	*Dasyatis chrysonota*	Blue stingray	*	*
Haemulidae	*Pomadasys commersonnii*	Spotted grunter	*	
Hemiramphidae	*Hemiramphus far*	Spotted halfbeak		*
Monodactylidae	*Monodactylus falciformis*	Cape moony	*	
Mugilidae	*Liza dumerili*	Groovy mullet	*	*
Mugilidae	*Liza richardsonii*	Southern mullet	*	*
Mugilidae	*Myxus capnesis*	Freshwater mullet		*
Pomatomidae	*Pomatomus saltatrix*	Bluefish		*
Sparidae	*Diplodus capensis*	Blacktail seabream	*	*
Sparidae	*Diplodus hottentotus*	Zebra seabream	*	*
Sparidae	*Lithognathus lithognathus*	White Steenbras	*	
Sparidae	*Rhabdosargus globiceps*	White stumpnose	*	*
Sparidae	*Rhabdosargus holubi*	Cape stumpnose	*	*
Sparidae	*Sarpa salpa*	Strepie	*	*
Tetraodontidae	*Amblyrhynchotes honckenii*	Evil eyed puffer fish	*	*
Other				
Phalacrocoracidae	*Phalacrocorax capensis*	Cape cormorant	*	
Sepiidae	*Sepia officinalis*	Common cuttlefish	*	