UNDERSTANDING RESOURCE PARTITIONING IN THREE SPECIES OF GOBIIDAE LIVING IN THE WARM-TEMPERATE SUNDAYS ESTUARY

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PHUMZA MALIBONGWE NDALENI

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“I am aware of the University’s Policy on Plagiarism. I have acknowledged all the people’s ideas, phrases, passages or illustrations which I included in this thesis.”

Name: Ndaleni Phumza Malibongwe

Student number: g15n6207

Signed:

ABSTRACT

Estuaries represent a transition zone where saline water combines with freshwater, and this results in temporal and spatial variability in both biotic and abiotic parameters. Because the environmental gradient changes so rapidly in areas and the estuarine environment is harsh, fish need to either be able to specialise to one particular suite of abiotic contexts or have the physiological mechanisms to allow them to move throughout the gradient of the estuary. Apart from this, other factors such as climate change, habitat degradation, over exploitation of resources and the introduction of alien species also negatively influence fish species living in estuaries. These anthropogenic stressors can cause species exclusion in areas which were previously suitable for species with particular suites of traits. Measuring resource partitioning within functional groups not only helps in determining the functional structure of communities but also explains how organisms use resources in a community. Resource partitioning studies provide a useful conceptual framework to understand species interactions in a community and identify major resource dimensions along which species segregate. This study aimed to describe resource partitioning among the Gobiidae species, as the family is well represented in South African estuaries (24 species), is highly abundant and has several species which co-exist in individual estuaries. To accomplish this, different forms of resource partitioning that promote the coexistence of the three most abundant species found in warm-temperate South African estuaries; the Knysna sand goby *Psammogobius knysnaensis* (Smith, 1935)*,* Prison goby *Caffrogobius gilchristi* (Boulenger, 1898) and River goby *Glossogobius callidus* (Smith, 1937) were investigated in the Sundays Estuary. This was accomplished by determining the reproductive biology, interspecific differences in feeding morphology, feeding ecology and habitat partitioning among the three species.

The distribution and abundance of potential prey in the estuary (mesozooplankton and macrozoobenthic communities) were investigated, with both mesozooplankton and macrozoobenthic communities grouping into three distinct communities along the environmental gradient. Feeding morphological traits responsible for prey location, chasing, capture, mastication, ingestion and digestion were investigated. Little morphological overlap was observed between *P. knysnaensis* and *C. gilchristi*, indicating little dietary overlap between these two species. In contrast, the feeding morphology of *Glossogobius callidus* overlapped with both *P. knysnaensis* and *C. gilchristi*, indicating the potential to compete with both species for food. The trophic ecology of the three species was investigated using stomach content analysis, whereby prey abundance, frequency of occurrence and percentage of volume were determined. For *P. knysnaensis*, the incorporation of cyclopoids and ostracods decreased with fish size and that of bivalves and amphipods increased. *Caffrogobius gilchristi* was found to be a specialist feeder on cyclopoids but as the species increased in size the incorporation of amphipods, mysids, brachyurans and gastropods increased. *Glossogobius callidus* was found to be a generalist feeder, feeding on cyclopoids, amphipods and chironomids. The incorporation of cyclopoids decreased with fish size and the consumption of large sized amphipods and chironomids increased with fish size. To assess their reproductive biology, gonadosomatic index (GSI) was used to study the breeding season. In all three species, reproduction peaked in spring and summer, and this is associated with peak phytoplankton and zooplankton biomass in estuaries. Length at 50% maturity and fecundity were also determined.

The three species predominated in different locations in the estuary, which explains why they were able to use similar resources and reproduce at the same time. *Psammogobius knysnaensis* was abundant in the sandy mouth region, with sediment type and prey (bivalves and mysids) availability affecting its distribution. *Caffrogobius gilchristi* was abundant in the muddy lower reaches of the estuary, with its distribution influenced by sediment composition and temperature. *Glossogobius callidus* was abundant in the turbid upper reaches, with its distribution influenced by prey (amphipods) availability and turbidity. Different habitat preferences and diet among the specific size ranges enabled coexistence among the species. This explains the high abundances and coexistence of gobies in South African estuaries.

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CHAPTER ONE

General introduction

Estuaries represent a transition zone where saline water combines with freshwater creating temporal and spatial variability in both biotic and abiotic parameters, with the scale of variability dependent on the size and type of estuary (Whitfield, 2019). Conditions within estuaries, particularly salinity, temperature and turbidity, vary with seasons, tides and freshwater flow (Whitfield, 2019). Since estuaries occur where freshwater mixes with seawater, they often exhibit high salinity variability. As such, salinity is the most notable driver of fish species distribution in estuaries, depending on species-specific salinity tolerances (Barletta et al., 2008; Whitfield, 2019) The most essential adaptation required for fishes to occur in estuaries is the ability to adjust to changing salinity regimes (Whitfield, 2015). Longitudinal salinity differences limit the distribution of fish species in estuaries with species occurring only within their preferrerd salinity ranges, unlike in fresh and marine environments where they can have a wider distribution (Barletta et al., 2008). Consequently, despite estuaries being highly productive environments, relatively few species are able to adapt to their variability, and estuaries typically have a lower species diversity than their adjacent marine and freshwater environments (Whitfield and Harrison, 2020).

Fish estuarine use categories

Fish species which use estuaries are divided into four estuarine use categories: marine, diadromous, freshwater and estuarine species, and within those categories are further divided into different estuarine use guilds (Potter et al., 2015; Figure 1.1; Table 1.1). The first category, marine species, spawn at sea and generally use estuaries for part of their life cycle. Within this category are marine stragglers, which are stenohaline species that enter estuaries in low numbers and are mostly recorded in the mouth region (Potter et al., 2015). There are also euryhaline marine estuarine-opportunist species, which use estuaries as juveniles but use coastal marine waters as alternative nursery areas (Potter et al., 2015). Euryhaline marine estuarine-dependent species enter estuaries as late-stage larvae and juveniles, and use estuaries as nursery areas before returning to the sea as sub-adults and adults (Potter et al., 2015). Juveniles of this group dominate the species richness within estuaries. The second category is comprised of catadromous fishes, which live in freshwater but return to sea to spawn

(Whitfield, 2019). The third category of fish occurring in estuaries are freshwater species, which breed in dams, lakes, rivers, streams and pans (Whitfield, 2019). Freshwater estuarine-opportunist species can extend beyond oligohaline sections, while freshwater stragglers spawn in freshwater, but can also occur in low saline areas of the estuary (Potter et al., 2015). The fourth category is comprised of highly euryhaline estuarine resident species, which are able to breed within estuaries, but some are also capable of breeding in both marine and freshwater environments (Whitfield, 2019).

The first guild within this category are solely estuarine fishes, which are only found in estuaries (Potter et al., 2015). Estuarine and freshwater species are represented by estuarine and freshwater populations, and estuarine and marine species are represented by estuarine and marine populations (Figure 1.1, Table 1.1). Estuarine migrant species spawn in estuaries, and may be washed out to sea as larvae and then return to the estuary at a later larval stage (Potter et al., 2015). Although estuarine resident species have low species diversity, they are highly abundant within estuaries (Harrison and Whitfield, 2008; Strydom, 2015).

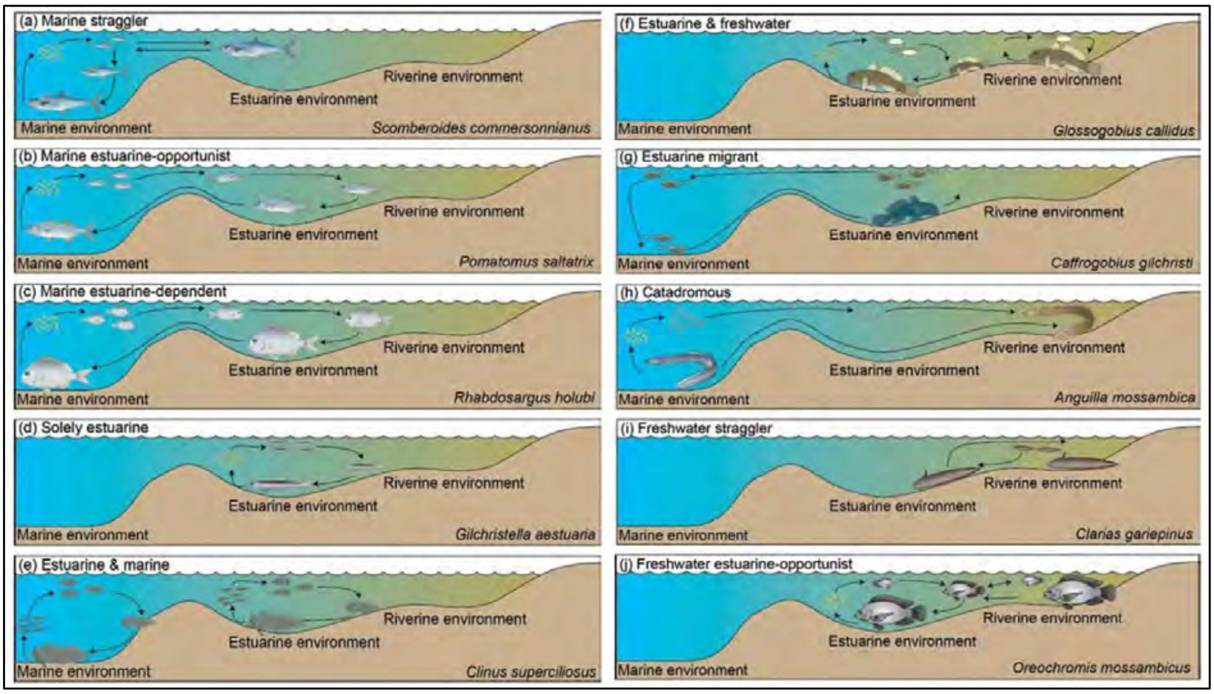


Figure 1.1: Guilds of fishes in South African estuaries (after Whitfield, 2019)

Table 1.1: Differences in estuarine use categories and guilds of South African estuary-associated fishes (after Potter et al., 2015 and Whitfield, 2019).

Category and guild	**Definition**	**Examples**
Marine category: species that spawn at sea		
Marine straggler	Typically enter estuaries sporadically and in low numbers and are most common in the lower reaches where salinities typically do not decline far below ~ 35. Often stenohaline	Leopard flounder (*Bothus pantherinus*), Sand steenbras (*Lithognathus mormyrus*), Barred flagtail (*Kuhlia mugil*)
Marine estuarine-opportunist	Regularly enter estuaries in substantial numbers, particularly as juveniles, but use, to varying degrees, coastal marine waters as alternative nursery areas	Bluefish (*Pomatomus saltatrix*), Flathead mullet (*Mugil cephalus*), Blacktail (*Diplodus capensis*)
Marine estuarine-dependent	Juveniles require sheltered estuarine habitats and are thus not present along exposed coasts where they spend the rest of their life	Cape stumpnose (*Rhabdosargus holubi*), White steenbras (*Lithognathus lithognathus*), Oval moony (*Monodactylus falciformis*)
Estuarine category: species with populations in which the individuals complete their life cycles within the estuary		
Solely estuarine	Found only in estuaries	Estuarine round herring (*Gilchristella aestuaria*), sharptail goby (*Oligolepis acutipennis*), Bigmouth goby (*Psammogobius bikolanus*)
Estuarine & marine	Also represented by marine populations	Sand goby (*Psammogobius knysnaensis*), Super klipfish (*Clinus superciliious*), Longsnout pipefish (*Syngnathu temmincki*)
Estuarine & freshwater	Also represented by freshwater populations	River goby (*Glossogobius callidus*), Checked goby (Redigobius dewaali), Barebreast goby (*Silhouettea sibayi*)
Estuarine migrant	Spawn in estuaries but may be flushed out to sea as larvae and later return at some stage to the estuary	Prison goby (*Caffrogobius gilchristi*)
Diadromous category: species that migrate between the sea and fresh water		
Catadromous	Spend their trophic life in fresh water and subsequently migrate out to sea to spawn	Shortfin eel (*Anguilla bicolor*), Giant mottled eel (*Anguilla marmorata*), Longfin eel (*Anguilla mossambica*)
Freshwater category: species that spawn in freshwater		
Freshwater straggler	Found in low numbers in estuaries and whose distribution is usually limited to the low salinity, upper reaches of estuaries	Banded tilapia (*Tilapia sparrmanii*), Cape galaxias (*Galaxias zebratus*)
Freshwater estuarine-opportunist	Found regularly and in moderate numbers in estuaries and whose distribution can extend well beyond the oligohaline sections of these systems	Mozambique tilapia (*Oreochromis mossambicus*), Checked goby (*Redigobius dewaali*), Tank goby (*Glossogobius giuris*)

Fish in estuaries can also be divided into feeding mode functional groups based on the primary method of feeding (Elliot et al., 2007; Table 1.2). Within South African estuaries all fish functional groups (estuarine use and feeding mode) decline in species richness between the tropical and temperate biogeographic regions, primarily as a result of temperature changes (Whitfield and Harrison, 2020).

Table 1.2: Feeding mode functional groups (adapted from Elliot et al. 2007)

Trophic category	**Examples**
Herbivores (Graze predominantly on living macroalgal and macrophyte material or phytoplankton)	*Sarpa salpa* and *Hyporhamphus capensis*
Detritivores (Feed predominantly on detritus and/or microphytobenthos)	*Chanos chanos* and *Oreochromis mossambicus*
Omnivores (Feed predominantly on filamentous algae, macrophytes, periphyton, epifauna and infauna	*Rhabdosargus holubi* and *Diplodus capensis*
Zooplanktivores (Feed predominantly on zooplankton e.g., hydroids, planktonic crustaceans, fish eggs/larvae)	*Hilsa kelee, Thryssa vitrirostris* and *Ambassis ambassis*
Zoobenthivores (Feed predominantly on invertebrates associated with the substratum, including animals that live just above the sediment (hyperbenthos), on the sediment (epifauna) or in the sediment (infauna)	*Pomadasys commersonnii, Pomadasys kaakan, Acanthopagrus vagus* and *Rhabdosargus sarba*
Piscivores (Feed predominantly on finfish but may include large nektonic invertebrates)	*Strongylura leiura, Caranx sexfasciatus, Elops machnata, Megalops cyprinoides, Pomatomus saltatrix, Argyrosomus japonicas* and *Sphyraena barracuda*
Opportunist (Feed on such a diverse range of food that they cannot be readily assigned to one of the above categories)	*Terapon jarbua*

Global change impacts on fish

Multiple pressures threaten this limited biodiversity in estuarine environments. Amongst these are climate change, habitat degradation, over exploitation of resources and the introduction of alien species (Chust et al., 2013). Since river flow affects the structure and functioning of fish species, perturbations in rainfall patterns due to climate change can result in disruptions to water quality, affecting fish distribution patterns (James et al., 2013). Flood events can also change sediment deposition in estuaries, further affecting the structure and functioning of benthic fish species in affected locations (James et al., 2013). Sea level rise due to climate change is another factor threatening fish species living in estuaries. An increase in sea level has the potential to change the hydrogeomorphology of estuaries, resulting in the loss of habitats such as salt marshes and mangroves, and this can affect fish communities (James et al., 2013). Thermal regimes are a major driver globally of species range distributions, and so any changes in water temperatures also affect fish distribution within estuaries. Warming of coastal and estuarine waters forces species to migrate to higher latitudes that better fit their thermal requirements (Whitfield et al., 2016).

Warming has also resulted in an increase in the occurrence of tropical fishes along the south east coast of South Africa (James et al., 2013). Such colonization by new groups of species can negatively affect species already living in these high latitudes, in what is referred to as climate facilitated biological invasion (Whitfield et al., 2016). Incipient colonisation of new habitats by warm-adapted species can negatively affect populations of native species through competition in resource-limited environments. Despite thermally induced migration dynamics, new habitats may not be suitable for an organism in terms of its physiological requirements or diet, which can negatively affect individual ecological functioning and therefore fitness. When new habitats do not provide enough food for energy investment this can result in stunted growth and failed reproduction (De Vlaming, 1971; Anderson, 1988; Persson and Ross, 2006).

Coastal environments tend to have higher human population pressure than inland areas (Whitfield et al., 2019). Intense human activities in these regions often result in increased pollution, habitat degradation and loss, as well as overfishing, which all negatively affect fish populations (Little et al., 2017; Vasconcelos et al., 2017; Whitfield et al., 2019). For example, citrus farming in the Sundays River Valley in the warm-temperate region of South Africa causes high nutrient deposition in the Sundays Estuary, resulting in low dissolved oxygen and eutrophic conditions in the middle reaches of the system (Lemley et al., 2018). Low dissolved oxygen and hypoxia may ultimately result in fish kills and major perturbations to the functioning of the environment, including habitat loss (Whitfield, 1995). Anthropogenic stressors, such as low dissolved oxygen and habitat destruction, may ultimately cause species exclusion in areas that were previously suitable for species with particular suites of traits. This is pronounced in aquatic environments such as estuaries and rivers, as they are considered to be biogeographic islands since they are separated by land (Cabral et al., 2001; Ram et al., 2014; Komoroske et al., 2016). In addition, changes in species assemblages and functionality are likely to be higher in estuaries than the marine and freshwater environments as the community assemblages often have a high abundance of estuarine species adapted to the stochastic nature of the habitat, which are more likely to suffer from stressor pressures (Whitfield and Harrison, 2020; Comte et al., 2021).

Resource partitioning

When species are functionally similar, and co-exist in a closed water body or an area with restricted movement, they are able to persist in sympatry through resource partitioning, by evolving traits which facilitate differential consumption of resources (niche partitioning) or indeed by responding differently to environmental heterogeneity (i.e., habitat partitioning), which then drives spatially explicit abundance patterns (Leibold and McPeek, 2006; Bolnick et al., 2007). Measuring resource partitioning within functional groups not only helps in determining the functional structure of communities but also explains how organisms use resources in a community (Da Silva et al., 2019). Resource partitioning studies provide a useful conceptual framework to 1) understand species interactions in a community and 2) identify major resource dimensions along which species segregate. Specialisation in resource use minimises competition, thereby enabling stable coexistence between similar competing species in aquatic systems (Guo et al., 2014). Specialisation in resource (food) use explains why multiple species are able to coexist in aquatic systems in the same habitats. Niche differentiation and neutral processes (used as a null model for testing the mechanisms of coexistence) play an important role in maintaining species diversity at different scales (Leibold and McPeek, 2006).

Dietary differences among species are mostly driven by morphological differences (Sibbing and Nagelkerke, 2001; Table 1.3). Specialisation in resource use minimizes competition, enabling coexistence between similar competing species, and may explain how multiple species are able to coexist in aquatic systems (Svanbäck and Bolnick, 2007; Guo et al., 2014). By determining the type and extent of resource partitioning in similar species it is possible to derive trait-based mechanisms (e.g., differences in diet, habitat and feeding morphology), which promote coexistence. For some species, resource competition is avoided by closely related species occupying different habitats. This habitat partitioning enables the species to feed on the same resources but from different habitats (Tilney and Hecht, 1990; Islam et al., 2006). However, when the habitat is disturbed species can be forced into a new habitat, which can compel them to compete for resources (Bostrom-Einarsson, 2016).

Table 1.3: Some of the review studies on resource partitioning in estuaries around the world

Region	**Taxa**	**Partitioning**	**Reference**
Southern Rhode Island, USA	*Menidia menidia* (Atherinopsidae) and *Menidia beryllina* (Atherinopsidae)	Habitat, reproduction, growth rate and food	Bengtson, 1984
Broälven Estuary, Sweden, Europe	*Pomatoschistus microps* (Gobiidae), *Gasterosteus aculeatus* (Gasterosteidae), *Pungitius pungitius* (Gasterosteidae),	Food	Thorman, 1982

	Anguilla anguilla (Anguillidae), *Gobius niger* (Gobiidae), *Pleuronectes platessa* (Pleuronectidae), *Platichthys flesus* (Pleuronectidae) and *Gobiusculus flavescens* (Gobiidae)		
Chesapeake Bay and its tributaries USA	*Morone saxatilis* (Moronidae), *Pomatomus saltatrix* (Pomatomidae) and *Cynoscion regalis* (Sciaenidae).	Food	Hartman and Brandt, 1995
Grays Harbor, Willapa Bay, Yaquina Bay and Coos Bay; Washington (USA)	*Pleuronectes vetulus* (Pleuronectidae), *Citharichthys sordidus* (Paralichthyidae), *Psettichthys melanostictus* (Pleuronectidae) and *Platichthys stellatus* (Pleuronectidae).	Habitat	Rooper et al., 2006
Murray Estuary, South Australia	*Atherinosoma microstoma* (Atherinidae), *Afurcagobius tamarensis* (Gobiidae) and *Hyperlophus vittatus* (Clupeidae)	Food	Hossain et al., 2017
Thames Estuary, UK, (Europe)	*Limanda limanda* (Pleuronectidae), *Platichthys flesus* (Pleuronectidae), *Pleuronectes platessa* (Pleuronectidae), *Solea solea* (Soleidae), *Clupea harengus* (Clupeidae), *Sprattus sprattus* (Clupeidae), *Merlangius merlangus* (Gadidae), *Trisopterus minutus* (Gadidae), *Trisopterus luscus* (Gadidae), *Dicentrarchus labrax* (Moronidae), *Osmerus eperlanus* (Osmeridae), *Gasterosteus aculeatus* (Gasterosteidae), *Agonus cataphractus* (Agonidae), *Syngnathus rostellatus* (Syngnathidae), *Pomatoschistus minutus* (Gobiidae) and *Anguilla Anguilla* (Anguillidae).	Habitat	Attrill and Power, 2004
Columbia Estuary, Washington (USA)	*Oncorhynchus tshawytscha* (Salmonidae) and *Gasterosteus aculeatus* (Gasterosteidae)	Food	Spilseth and Simenstad, 2011
Paraíba do Norte Estuary, Brazil (South America)	*Sciades herzbergii* (Ariidae), *Sciades parkeri* (Ariidae), *Centropomus parallelus* (Perciformes), *Centropomus undecimalis* (Centropomidae), *Lutjanus alexandrei* (Lutjanidae), *Lutjanus jocu* (Lutjanidae), *Diapterus rhombeus* (Gerreidae), *Eucinostomus argenteus* (Gerreidae) and *Bathygobius soporator* (Gobiidae)	Food	Garcia and Vendel, 2016
Tubarão Estuary, Brazil (South America)	*Sphoeroides greeleyi* (Tetraodontidae) and *Sphoeroides testudineus* (Tetraodontidae)	Food and habitat	Lima et al., 2018

Cattle Creek and Gentle Annie Creek mangrove estuaries, Australia	*Acanthopagrus australis* (Sparidae) and *Acanthopagrus pacificus* (Sparidae)	Food	Sheaves et al., 2014
Tubarão Estuary, Brazil (South America)	*Lutjanus alexandrei* (Lutjanidae), *Lutjanus analis* (Lutjanidae), *Lutjanus jocu* (Lutjanidae) and *Lutjanus synagris* (Lutjanidae)	Habitat and food	Lustosa-Costa et al., 2020
(Sundays, Swartkops, Krom and Gamtoos), South Africa (Africa)	*Argyrosomus hololepidotus* (Sciaenidae), *Elops machnata* (Elopidae), *Lichia amia* (Carangidae), *Platycephalus indicus* (Platycephalidae), *Pomadasys commersonni (Haemulidae), Tachysurus feliceps* (Bagridae) and *Monodactylus falciforrnis* (Monodactylidae)	Food	Marais, 1984
St. Croix Estuary, Canada	*Gasterosteus aculeatus* (Gasterosteidae), *Gasterosteus wheatlandi* (Gasterosteidae), *Pungitius pungitius* (Gasterosteidae) and *Apeltes quadracus* (Gasterosteidae)	Food	Delbeek and Williams, 1987
Great Fish Estuary, South Africa, Africa	*Glossogobius callidus* (Gobiidae) and *Redigobius dewaali* (Gobiidae)	Food and morphology	Wasserman, 2012
Tama Estuary, Japan (Isia)	*Lateolabrax japonicas* (Lateolabracidae), *Acanthogobius flavimanus* (Oxudercidae) and *Mugil cephalus* (Mugilidae)	Food	Kon et al., 2008
Pahang Estuary, Malaysia (Isia)	Pristigasteridae, Engraulidae, Pangasiidae, Cyprinidae, Scieanidae, Mugilidae, Polynemidae, Latidae, Osphronemidae, Eleotridae, Scatophagidae, Ariidae, Plotosidae and Clupeidae,	Food and habitat	Azfar et al., 2015
Mamanguape Estuary, Brazil (South America)	*Rhinosardinia bahiensis* (Clupeidae), *Anchovia clupeoides* (Engraulidae), *Lycengraulis grossidens* (Engraulidae), *Hyporhamphus unifasciatus* (Hemiramphidae), *Atherinella brasiliensis* (Atherinopsidae), *Centropomus undecimalis* (Centropomidae), *Caranx latus* (Carangidae), *Oligoplites saurus* (Carangidae), *Trachinotus goodei* (Carangidae), *Lutjanus synagris* (Lutjanidae) *Eugerres brasilianus* (Gerreidae), *Menticirrhus littoralis* (Sciaenidae), *Bathygobius soporator* (Gobiidae), *Gobionellus oceanicus* (Oxudercidae), *Citharichthys spilopterus* (Paralichthyidae), *Achirus lineatus*	Habitat, diet and ecomorphological indices	Pessanha et al., 2015

	(Achiridae) and *Sphoeroides testudineus* (Tetraodontidae)		
Mamanguape estuary, Brazil (South America)	*Eucinostomus melanopterus* (Gerreidae), *Eugerres brasilianus* (Gerreidae) and *Diapterus rhombeus* (Gerreidae)	Habitat	Araújo et al., 2016
Mondego Estuary, Portugal (Europe)	*Dicentrarchus labrax* (Moronidae), *Platichthys flesus* (Pleuronectidae) and *Solea solea* (Soleidae)	Habitat	Martinho et al., 2007
United States	Red drum *Sciaenops ocellatus* and spotted seatrout *Cynoscion nebulosus*	Food	Livernois, 2015
Walpole-Nornalup Estuary, Australia	*Acanthopagrus butcheri* (Sparidae), *Rhabdosargus sarba* (Sparidae) and *Chrysophrys auratus* (Sparidae)	Food	Stewart, 2018
Davis Bayou, Mississippi (USA)	*Lepomis miniatus* (Centrarchidae), *Lepomis microlophus* (Centrarchidae) and *Lepomis macrochirus* (Centrarchidae)	Habitat and food	Vanderkooy, 2000
Belongil Creek Estuary and Tallows Creek Estuary, Australia	*Acanthopagrus australis* (Sparidae), *Platycephalus fuscus* (Platycephalidae), *Sillago ciliata* (Sillaginidae) and *Mugil cephalus* (Mugilidae)	Food	Hadwen et al., 2007
Various estuaries, Columbia (USA)	*Fundulus heteroclitus* (Fundulidae), *Fundulus majalis* (Fundulidae), *Fundulus diaphanus* (Fundulidae) and *Fundulus lucia* (Fundulidae)	Habitat	Weisberg, 1986
Goiana Estuary, Brazil (South America)	*Cathorops spixii* (Ariidae) and *Cathorops agassizii* (Ariidae)	Food	Dantas et al., 2013
Nakdong Estuary, Korea (Isia)	*Pennahia argentata* (Sciaenidae) and *Sillago japonica* (Sillaginidae)	Food	Huh et al., 2018
Wilson Inlet, Australia	*Leptatherina wallacei* (Atherinidae), *Leptatherina presbyteroides* (Atherinidae), *Atherinosoma elongate* (Atherinidae), *Pseudogobius olorum* (Gobiidae), *Favonigobius suppositus* (Gobiidae) and *Favonigobius lateralis* (Gobiidae)	Habitat and food	Humphries and Potter, 1993
Various estuaries around Everglades National Park, Florida (USA)	*Sciaenops ocellatus* (Sciaenidae) and *Centropomus undecimalis* (Centropomidae)	Food and habitat	Malinowski et al., 2019
Venice Lagoon, Italy (Europe)	*Zosterisessor ophiocephalus* (Gobiidae), *Gobius niger* (Gobiidae), *Gobius cobitis* (Gobiidae), *Pomatoschistus marmoratus*	Reproduction and habitat	Malavasi et al., 2005

	(Gobiidae), *Pomatoschistus minutus* (Gobiidae), *Pomatoschistus canestrinii* (Gobiidae) and *Knipowitschia panizzae* (Gobiidae)		
Tijuana Estuary, California (USA)	*Fundulus parvipinnis* (Fundulidae), *Atherinops affinis* (Atherinopsidae), *Leptocottus armatus* (Cottidae), *Clevelandia ios* (Oxudercidae), *Gillichthys mirabilis* (Gobiidae), *Paralichthys californicus* (Paralichthyidae) and *Hypsopsetta guttulata* (Pleuronectidae)	Food	West et al., 2003
Wilson Inlet, Australia	*Mugil cephalus, Aldrichetta forsteri, Sillaginodes punctata, Arripis georgiana, Cnidoglanis macrocephalus* and *Platycephalus speculator*	Food and feeding morphology	Plattel et al., 2006
Cross Estuary, Nigeria	*Labeo senegalensis* and *Pseudotolithus elongates*	Food and feeding morphology	Udoh and Ekpo, 2017
Rio Mamanguape Estuary, Brazil	*Rhinosardinia bahiensis, Anchovia clupeoides, Lycengraulis grossidens, Hyporhamphus unifasciatus, Atherinella brasiliensis, Centropomus undecimalis, Caranx latus, Oligoplites sauru, Trachinotus goodie, Lutjanus synagris, Eugerres brasilianus, Menticirrhus littoralis, Bathygobius soporator, Gobionellus oceanicus, Citharichthys spilopterus, Achirus lineatus* and *Sphoeroides testudineus*	Food and feeding morphology	Pessanha et al., 2015

Gobiidae review

The Gobiidae family provides excellent subjects to study resource partitioning among the estuarine resident and zoobenthivore functional groups in estuaries. Gobiidae is the largest teleost family globally, with more than 2000 species and 200 genera worldwide (Thacker and Roje, 2011). The family is recorded in tropical, subtropical and temperate seas and rivers (Tutman et al., 2020). Gobies are small benthic fishes with habitats ranging from marine, brackish to freshwater environments, although the majority of species are marine (Tutman et al., 2020). Trophic categories found within the family include zoobenthivores and zooplanktovores (Bakun and Weeks, 2006; Lönnstedt et al., 2013; McNeish et al., 2018). Gobiidae are abundant in the subtropical and temperate regions of southern Africa (Whitfield, 1999). They are one of the dominant fish families occurring in South African estuaries in terms of richness and abundance (Harrison and Whitfield, 2006; James et al., 2007; Strydom, 2015), with the estuarine resident and zoobenthivorous guilds in South African estuaries dominated (in terms of species richness and abundance in benthic habitats) by members of the Gobiidae family. Gobies also play a critical role in the trophic web within detritus-generated food chains in South African estuaries (Whitfield, 2019, Table 1.4).

Table 1.4: Food chains of southern African estuarine fishes (Whitfield 2019).

Detritus-generated food chains				
Detritus →	Benthos (epifauna) →	Benthophagous fishes (e.g., *Sillago sihama*)		
Detritus →	Benthos (infauna) →	Benthophagous fishes (e.g., *Pomadasys commersonnii*)	→	Large predators (e.g., *Carcharinus leucas*)
Detritus →	Small benthos →	Larger invertebrates and small benthic fishes (e.g., *Glossogobius callidus*)	→	Large fishes (e.g., *Lutjanus argentimaculatus*)
Detritus →	Detritivorous fishes (e.g., *Mugil cephalus*)			
Detritus →	Macrobenthos →	Large predators (e.g., *Himantura leoparda*)		
Detritus →	Micronckton →	Intermediate predators (e.g., *Ambassis dussumieri*)	→	Large predators (e.g., *Sphyraena barracuda*)
Detritus →	Zooplankton →	Small fishes and invertebrates	→	Larger fishes (e.g., *Caranx sexfasciatus*)

In South African estuaries the family is represented by 24 species, with their species numbers decreasing from the subtropical, warm temperate and cool temperate region respectively, as shown in Table 1.5 (Whitfield, 2019). Indeed, they are one of only two families (the other being Mugilidae) that are well represented in all South African estuaries (James et al., 2016). Within South African estuaries they are one of the most abundant benthic fish families (Richardson et al., 2006; Bailey and James, 2013; Nodo et al., 2017; Nodo et al., 2018). The species composition within the family decreases from the subtropical to cool-temperate regions (Whitfield, 2019).

Table 1.5: Gobiidae species occurring in South African estuaries, by guild (E & F = estuarine and freshwater; EM = estuarine migrant, E & M = estuarine and marine, FEO = freshwater estuarine opportunist, SE = solely estuarine) and biogeographic region (S = subtropical, W = warm-temperate, C = cool-temperate) (after Whitfield 2019). Common species are shown in bold.

Species	**Fish guild**	**Region**	**IUCN Red List Status**
Favonigobius melanobranchus	E & F	S	Lower Risk: near threatened
Favonigobius reichei	E & F	S	Least Concern
Glossogobius callidus	**E & F**	**S/W**	**Least Concern**
Redigobius dewaali	E & F	S/W	Least Concern
Silhouettea sibayi	E & F	S	Endangered
Caffrogobius gilchristi	**EM**	**S/W/C**	**Least Concern**
Caffrogobius natalensis	E & M	S/W	Least Concern
Caffrogobius nudiceps	E & M	W/C	Least Concern
Paratrypauchen microcephalus	E & M	S	Least Concern
Psammogobius knysnaensis	**E & M**	**S/W/C**	**Least Concern**
Taenioides esquivel	E & M	S	Not Evaluated
Trypauchenopsis intermedia	E & M	S	Least Concern
Yongeichthys nebulosus	E & M	S	Least Concern
Awaous aeneofuscus	FEO	S	Least Concern
Glossogobius giuris	FEO	S	Least Concern
Croilia mossambica	SE	S	Least Concern
Oligolepis acutipennis	SE	S	Least Concern
Oxyurichthys keiensis	SE	S/W	Least Concern
Oxyurichthys ophthalmonema	SE	S	Least Concern
Pandaka silvana	SE	S/W	Not Evaluated
Periophthalmus argentilineatus	SE	S	Least Concern
Periophthalmus kalola	SE	S	Least Concern
Glossogobius biocellatus	SE	S	Least Concern
Redigobius bikolanus	SE	S	Least Concern

Within temperate estuaries, three of the most abundant gobies are the Knysna sand goby *Psammogobius knysnaensis* (Smith, 1935)*,* Prison goby *Caffrogobius gilchristi* (Boulenger, 1898) and River goby *Glossogobius callidus* (Smith, 1937) (James et al., 2007).

Psammogobius knysnaensis is endemic to southern African estuaries, ranging from Port Nolloth to KwaZulu Natal (Whitfield, 2019) and is abundant in the sandy lower reaches of South African estuaries (Richardson et al., 2006; Bailey and James, 2013; Nodo et al., 2018).

Psammogobius knysnaensis matures at approximately 30 mm TL, with reproduction occurring in spring and summer (Bennett, 1989). During reproduction, eggs are laid on hard surfaces such as rocks (Wasserman et al., 2017). After hatching fish larvae are carried by the ebb tide out to sea, and return to the estuary as post larvae (Whitfield, 1989). In situations where the estuary is closed larvae develop within the estuary, but this results in low adult numbers (Wasserman et al., 2017). Salinity tolerance of this species ranges from 2–35‰ (Whitfield, 2019). Prey items present in its diet include amphipods, polychaetes, insect larvae, isopods, copepods, cumacea, decapods and ostracods (Bennett, 1989, Whitfield, 1988).

Caffrogobius gilchristi (EM) is endemic to South African estuaries, occurring from the Olifants system on the west coast to Durban Bay (Whitfield, 2019). The species is associated with muddy habitats located in the middle or lower reaches of estuaries (McGregor et al., 2018; Nodo et al., 2018). In the Nahoon, Gonubie, Qora and Xhora estuaries, *C. gilchristi* is highly abundant in muddy rocky habitats (McGregor et al., 2018). In the Mbashe Estuary during a period where the estuary was characterised by high mud content, *C. gilchristi* was recorded in all reaches of this muddy estuary (James et al., 2020). The species matures at less than 50 mm TL during spring and summer (Bennett, 1989). They are adapted to breeding in estuaries by producing sticky eggs which become attached to submerged objects such as stones and shells (Whitfield, 2019). After hatching, their larvae are washed out to sea during the ebb tide, with post larvae returning to the estuary to become permanent residents in the estuary (Whitfield, 2019). The species forages at dawn and dusk (Bennett and Branch, 1990). Prey items present in their diet include copepods, mysids, crabs, gastropods, amphipods, chironomid larvae, anomurans, polychaetes, macrurans and small fishes (Whitfield, 1988; Bennett and Branch, 1990). Both prey availability and seasonal changes have an impact on the diet of this species (Whitfield, 1988; Bennett and Branch, 1990).

Glossogobius callidus (E and F) is endemic to coastal rivers and estuaries of southern Africa (Whitfield, 2019). The distribution of this species ranges from Mozambique to the Swartvlei region of the Western Cape (Whitfield, 2019). The species is mostly recorded in the saline upper and middle reaches of estuaries (Ter Morshuizen and Whitfield, 1994). The species starts to reproduce at 35 mm, with reproduction occurring in spring and summer (Boullé, 1989) and has a lifespan of about seven years (Mofu, 2016). During reproduction, eggs are laid on hard surfaces and rocks (Wasserman, 2015). During the nesting period, males provide parental care by guarding the eggs the whole incubation period, fanning them and protecting them from predators until they hatch (Wasserman, 2015). Amphipods, cyclopoids and chironomid larvae dominate the diet of this species in the early life-stages (Vumazonke, 2008; Wasserman, 2012).

Aims and objectives

Although there have been studies on the morphology, diet, reproduction and distribution of gobies in estuaries, a comprehensive resource partitioning study is lacking to fully understand the coexistence (and success) of goby species in South African estuaries. This study aims to describe resource partitioning and co-existence of the three most abundant gobies (Knysna sand goby *Psammogobius knysnaensis* (Smith, 1935), Prison goby *Caffrogobius gilchristi* (Boulenger, 1898) and River goby *Glossogobius callidus* (Smith, 1937) in the warm-temperate Sundays Estuary, South Africa. These species were selected since they are the dominat species within the Gobiidae family in temperate South African estuaries (James et al., 2007). This was accomplished by determining the following for the three species:

- Reproductive biology
- Interspecific differences in feeding morphology
- Feeding ecology
- Habitat partitioning

Thesis structure

This thesis is divided into seven chapters. The chapters cover a general introduction (Chapter 1), study area (Chapter 2), reproductive biology of the study species (Chapter 3), interspecific differences in feeding morphology (Chapter 4), feeding ecology of the study species (Chapter 5), distribution, abundance and habitat partitioning (Chapter 6) and a general discussion (Chapter 7).

CHAPTER TWO

Study area

Introduction

The Sundays Estuary is a permanently open estuary flowing into Algoa Bay in the Indian Ocean at 33.72^{0}S, 25.42^{0}E, 35 km north-east of Gqeberha, South Africa (Figure 2.1). The 310 km river rises in the Karoo (south central South Africa) and has a catchment area of 20 729 km (Beckley, 1984). The estuary is channel-like, and is approximately 21 km long (Baird, 2001; Scharler and Baird, 2005). The average depth of the estuary is four meters; the channel in the lower and middle reaches is approximately five meters in depth, while the upper reaches are shallower (± 2 m) (Whitfield and Harrison, 1996; Baird, 2001). Surface area is approximately three km^2 (Baird, 2001). The estuary's widest point is near the mouth and is approximately 800 m, from where it narrows to 20 m at the head of the estuary (Marais, 1981; Whitfield and Harrison, 1996).

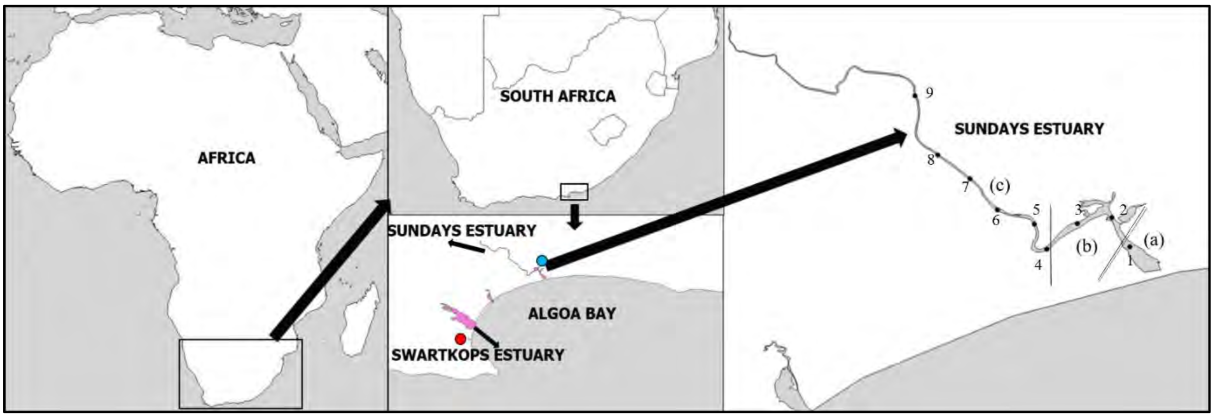


Figure 2.1: Sampling sites within the Sundays Estuary, Eastern Cape, South Africa. Red dot = Gqeberha, blue dot = Colchester, (a) = Euhaline, (b) = polyhaline and (c) = mesohaline region

The estuary is characterised by steep banks with little marginal vegetation and the absence of seagrass and large mud flats (Beckley, 1984; Lee et al., 2013). The pondweed *Potamogeton crispus* is common around the head of the estuary (Harrison and Whitfield, 1990). Along the littoral zone of the middle and upper reaches of the estuary, widespread beds of the common reed *Phragmites australis* are observed (Adams and Bate, 1999). In the middle reaches marginal vegetation is dominated by filamentous algal mats comprised of the *Vaucheria* species (Beckley, 1984; Kotsedi et al., 2012).

Although there is extensive citrus farming in the catchment of the river, there is little human habitation along both the river and estuary (Baird, 2001). The citrus farming results in high levels of nutrients within the estuary (Van Vuuren et al., 2015). As such, this estuary is characterised by high chlorophyll-a levels in the middle and upper reaches (Emmerson 1989; Hilmer, 1990; Hilmer and Bate, 1990; Kotsedi et al., 2012).

Freshwater inflow is relatively high, with continuous freshwater inflow via an inter-basin water transfer scheme (Perch et al., 1995). During the winter dry season and summer wet season freshwater inflow to the estuary ranges between 1.00 $m^3.s^{-1}$ and 2.74 $m^3.s^{-1}$, respectively (Baird, 2001). The estuary is highly stratified (vertical and horizontal) during neap tides, and becomes completely mixed during spring tides (Wooldridge and Bailey, 1982; Mackay and Schunann, 1990). It has a tidal prism of 1.5 m near the mouth and 0.75 m near the estuary head (Wooldridge and Bailey, 1982; Mackay and Schurnann, 1990).

Nine sampling sites from the euhaline to mesohaline region of estuary were sampled twice per season between January 2018 and September 2019 (Figure 2.1). The sampling sites were 2 km apart, except for the uppermost site which was 3 km from site eight.

Climatic conditions

Air temperature and wind

The Sundays Estuary is situated in the warm-temperate region. Westerly winds are common throughout the year, but a southerly shift occurs in winter (Goschen and Schumann, 2010). These winds reach their maximum speed and frequency in October and November, and their minimums are reached in May, June and July (Goschen and Schumann, 2010). During the study period (2018–2019) the minimum air temperature recorded was 10.1°C (9 August 2018) and the maximum was 31.1°C (18 December 2018) (Figure 2.2).

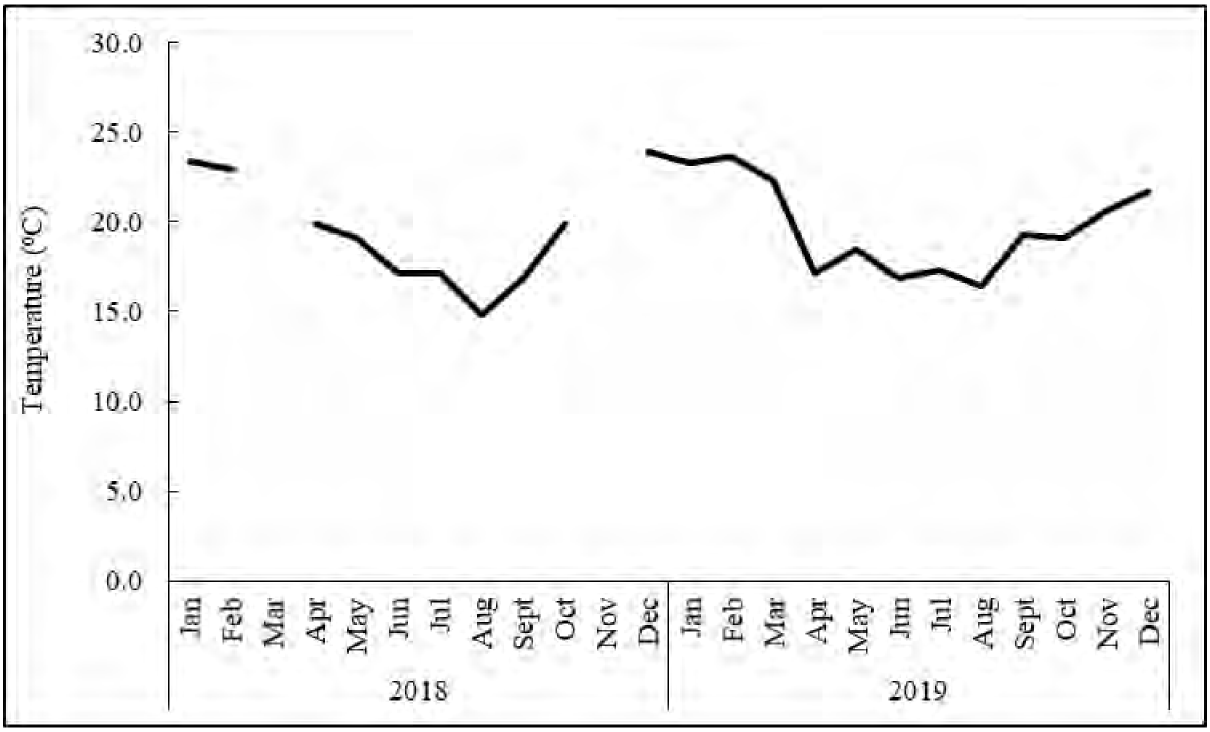


Figure 2.2: The average monthly air temperatures measured at Colchester in 2018–2019 (South African Weather Service)

Rainfall

In the south-eastern coastal region, rainfall patterns are influenced by the proximity and temperature of the Agulhas Current (Jury et al., 1992). Zengeni et al. (2016) showed that specifically around the Port Elizabeth region, high rainfall is common in summer and spring. This estuary receives a mean annual rainfall of approximately 323 mm and mean annual runoff of approximately 200 X 10^6 m^3 (Whitfield and Harrison, 1996). The study period coincided with a prolonged six-year drought in the region, with maximum rainfall recorded in September 2018 (67.2 mm) and the lowest rainfall in March and April 2018 (0 mm) (Figure 2.3).

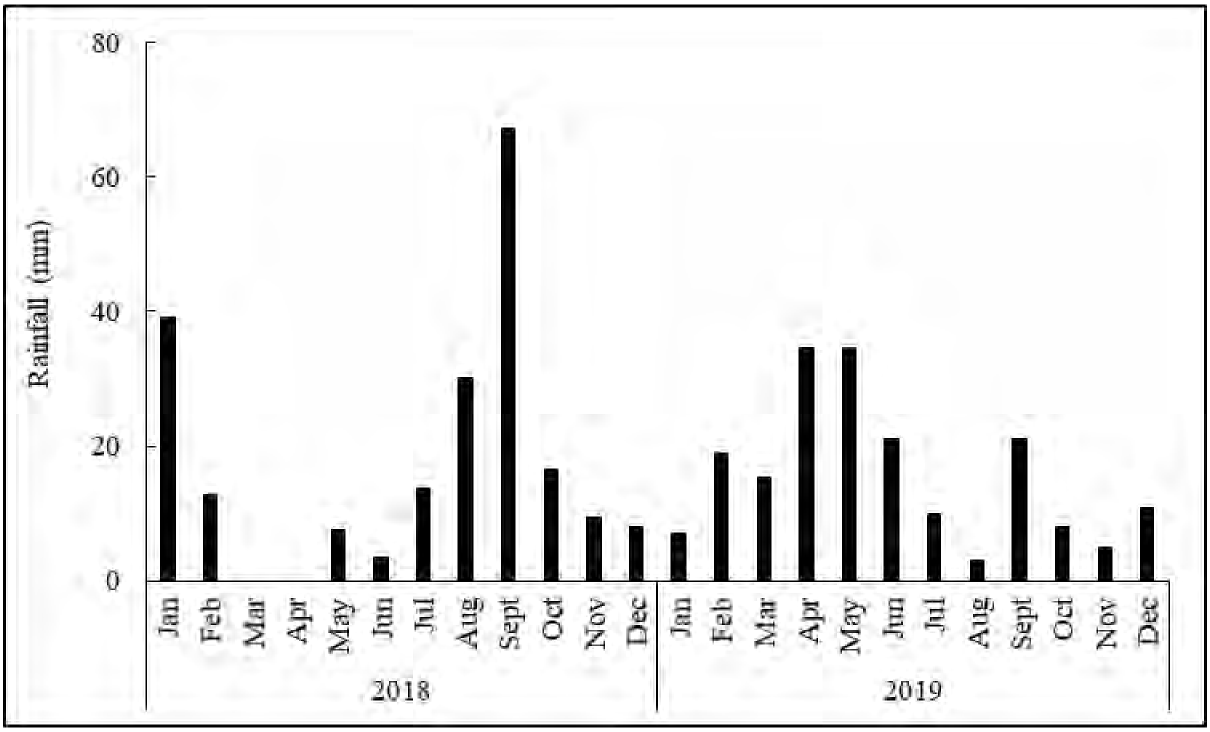


Figure 2.3: The average monthly rainfall measured at Colchester between 2018–2019 (South African Weather Service).

Abiotic characteristics of the Sundays Estuary

Abiotic parameters were measured at nine fixed sites situated along the length of the estuary twice per season between March 2018 and February 2019 (Figure 2.1). Measurements included temperature (°C), salinity (‰), pH, dissolved oxygen (mg/l) and turbidity (NTU). This sampling was conducted during daytime (08:00–17:00) at two water levels (bottom and surface). All measurements were taken using a YSI (6290) multi-parameter probe. At each site, sediments were collected once per season (one replicate) using a cone dredge for organic content analysis. Sediments were also collected at each site (one replicate) in March 2020 using a cone dredge for sediment particle size analysis. After collection in the field, sediments were stored in a cooler box with ice for later analysis in the SAEON Ocean Science laboratory (Nelson Mandela University).

In order to calculate sediment organic content, each sediment sample was subsampled to ~ 20 g, placed in a crucible of known weight and heated to dry in an oven at 65°C for 24 hours. The dry sediment was then weighed to obtain the dry weight. The subsample was then heated to 550°C in the furnace for eight hours, and then weighed to obtain the ash-free dry weight. From these measurements, the percentage of dry weight was then calculated using the equation:

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

A standard dry sieving method was used to determine sediment particle size (Gartzia-Bengoetxea et al., 2009). Wet sediment was dried in the oven at 20˚C for 24 h to remove all moisture. A King Test VB 200/300 Sieve Shaker was used to conduct grain size analysis. Mesh sizes used were 2000 µm (very coarse sand), 1000 µm (coarse sand), 500 µm (medium sand), 355 µm (medium sand), 250 µm (fine sand), 180 µm (fine sand), 125 µm (very fine sand), 90 µm (very fine sand), 63 µm (silt) and < 63 µm (clay). These mesh sizes were arranged in decreasing mesh size with the pan at the bottom. A total of 50 g of sediment was weighed from the dried sediment, poured on the top sieve and closed. Each sample was then sieved for 5 minutes. The remaining material from each sieve was then emptied onto a tray. Sediments from each tray were weighed and the percentage particle size contribution calculated using the equation:

$$Particle\ size\ \% = \frac{Sediment\ weight\ from\ each\ tray}{Total\ sediment\ weight} \times 100$$

Mean bottom and surface abiotic parameters recorded during the study period in each station are presented in Table 2.1.

Table 2.1: Mean (±SD) surface and bottom abiotic measurements for each station along the length of the Sundays Estuary from March 2018 to February 2019. S = surface and B = bottom

Sampling sites	**Water level**	**Temperatures (^{0}C)**	**pH**	**Salinity (‰)**	**Turbidity (NTU)**	**Dissolved oxygen (mg/l)**
1 (2 km)	S	19.55 (±5.14)	8.46 (±0.72)	27.58 (±11.72)	3.15 (±2.00)	7.49 (±10.44)
	B	18.97 (±6.40)	8.47 (±0.62)	30.98 (±6.16)	4.14 (±5.10)	8.01 (±1.30)
2 (4 km)	S	20.11 (±8.51)	8.41 (±0.83)	20.99 (±8.16)	5.11 (±5.10)	8.63 (±5.66)
	B	20.80 (±16.05)	8.39 (±0.92)	29.66 (±9.36)	6.77 (±7.00)	7.89 (±2.26)
3 (6 km)	S	20.34 (±9.44)	8.41 (±0.85)	17.41 (±12.50)	5.36 (±9.10)	7.14 (±11.96)
	B	18.91 (±6.17)	8.3 (±0.88)	27.27 (±14.13)	9.13 (±12.30)	6.93 (±7.91)
4 (8 km)	S	20.14 (±8.90)	8.45 (±0.68)	14.42 (±11.23)	6.05 (±12.50)	8.51 (±6.77)
	B	19.22 (±7.47)	8.30 (±0.65)	24.35 (±20.89)	9.53 (±13.30)	6.12 (±5.85)
5 (10 km)	S	19.90 (±8.66)	8.45 (±0.62)	10.88 (±8.71)	10.26 (±25.70)	8.25 (±5.44)
	B	19.83 (±7.76)	8.37 (±0.71)	16.88 (±9.41)	10.46 (±19.00)	6.80 (±9.70)
6 (12 km)	S	19.68 (±8.82)	8.55 (±0.66)	7.46 (±7.40)	8.06 (±26.50)	6.53 (±2.84)
	B	19.74 (±9.06)	8.29 (±0.43)	13.63 (±19.98)	10.90 (±18.20)	4.95 (±5.87)
7 (14 km)	S	20.01 (±12.55)	8.50 (±0.45)	4.18 (±4.44)	11.29 (±21.10)	5.74 (±8.36)
	B	20.37 (±10.48)	8.4 (±0.59)	7.40 (±16.52)	17.64 (±25.40)	5.93 (±2.70)
8 (16 km)	S	19.99 (±10.68)	8.47 (±0.51)	5.77 (±22.93)	11.42 (±21.40)	6.63 (±5.07)
	B	17.15 (±21.61)	8.44 (±0.30)	6.76 (±23.12)	11.98 (±25.00)	6.4 (±4.92)
9 (21 km)	S	16.83 (±21.53)	8.28 (±0.82)	5.40 (±23.32)	11.03 (±20.50)	6.13 (±4.62)
	B	14.99 (±22.41)	8.31 (±1.24)	5.42 (±23.23)	11.58 (±27.80)	6.67 (±4.79)

During the study period, surface and bottom water temperatures decreased with distance from the mouth, with mean surface temperature decreasing from 19.55°C at the mouth to 16.83°C at the head of the estuary. Mean bottom water temperatures decreased from 20.80°C to 14.99°C. Horizontal and vertical salinity gradients were observed, with mean surface salinity decreasing from 27.58‰ at the mouth to 5.40‰ at the head of the estuary. Mean bottom salinity ranged from 30.98‰ (mouth) to 5.42‰ (head of the estuary) (Table 2.1). During the survey period, the salinity profile of this estuary reflected low freshwater input as the oligohaline region (0.5–4.9‰) was absent. The estuary was divided into three salinity regions according to the Venice System (Figure 2.1). These regions are based on mean bottom salinities as euhaline (30.0–39.9‰), polyhaline (18.0–29.9‰) and mesohaline (5.0–17.9‰) regions (Figure 2.1).

Mean bottom pH ranged from 8.30 (Sites 3 and 4) to 8.47 (Site 1) and surface pH from 8.28 (Site 9) to 8.55 (Site 6). Dissolved oxygen was lowest in the middle reaches of the estuary, with mean bottom water dissolved oxygen ranging from 4.95 (Site 6) to 7.89 mg/l (Site 2) and surface water dissolved oxygen from 5.74 (Site 7) to 8.63 mg/l (Site 2). Mean bottom water turbidity ranged from 4.14 (Site 1) to 17.64 (Site 7) NTU and mean surface turbidity from 3.15 (Site 1) to 11.42 NTU (Site 8).

In all seasons, organic content increased from the mouth (Site 1) to the middle reaches (Site 5 = 10 km from the mouth) and then decreased towards the uppermost site (Figure 2.4).

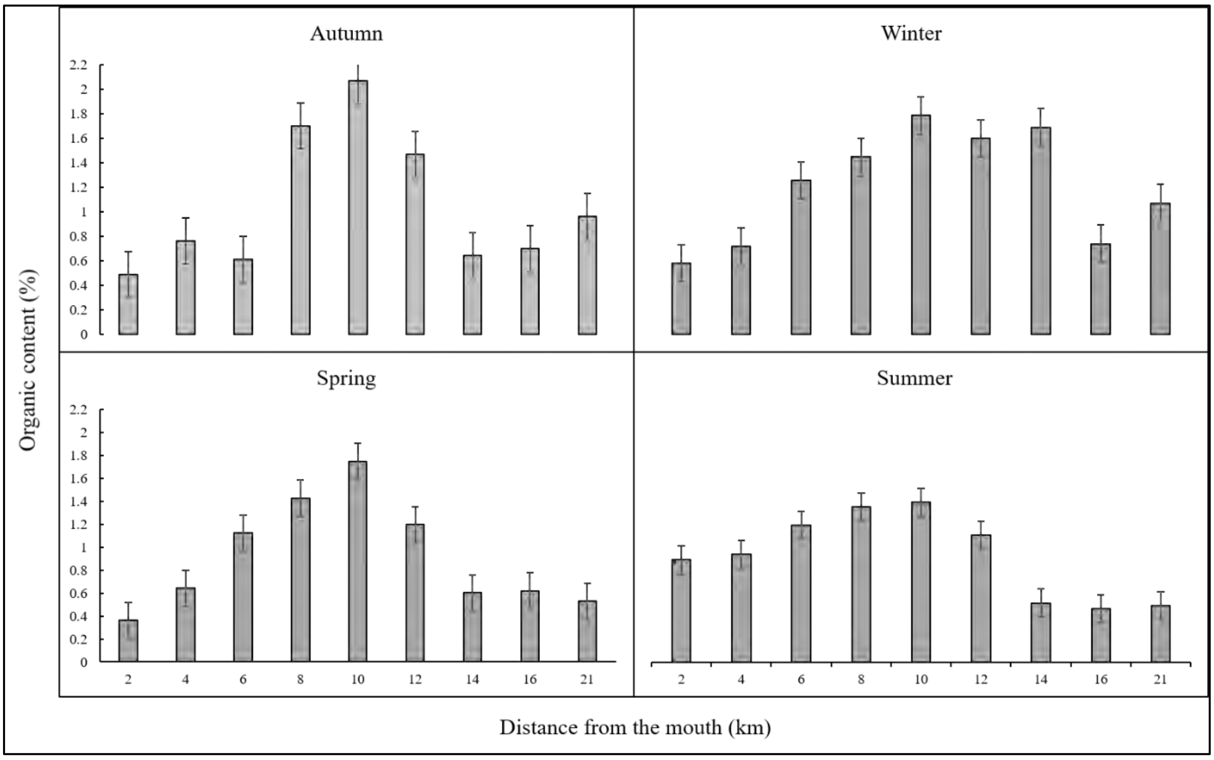


Figure 2.4: Organic content of sediment with distance from the mouth in the Sundays Estuary

Mean grain size increased with distance from the mouth and was highest at the uppermost site (Figure 2.5). Grain size contribution changed along the length of the estuary, with clay and very fine sand decreasing with distance from the mouth. The contribution of fine sand and medium sand increased with distance from the mouth. Fine sand had the highest contribution overall followed by medium and very fine sand (Figure 2.6).

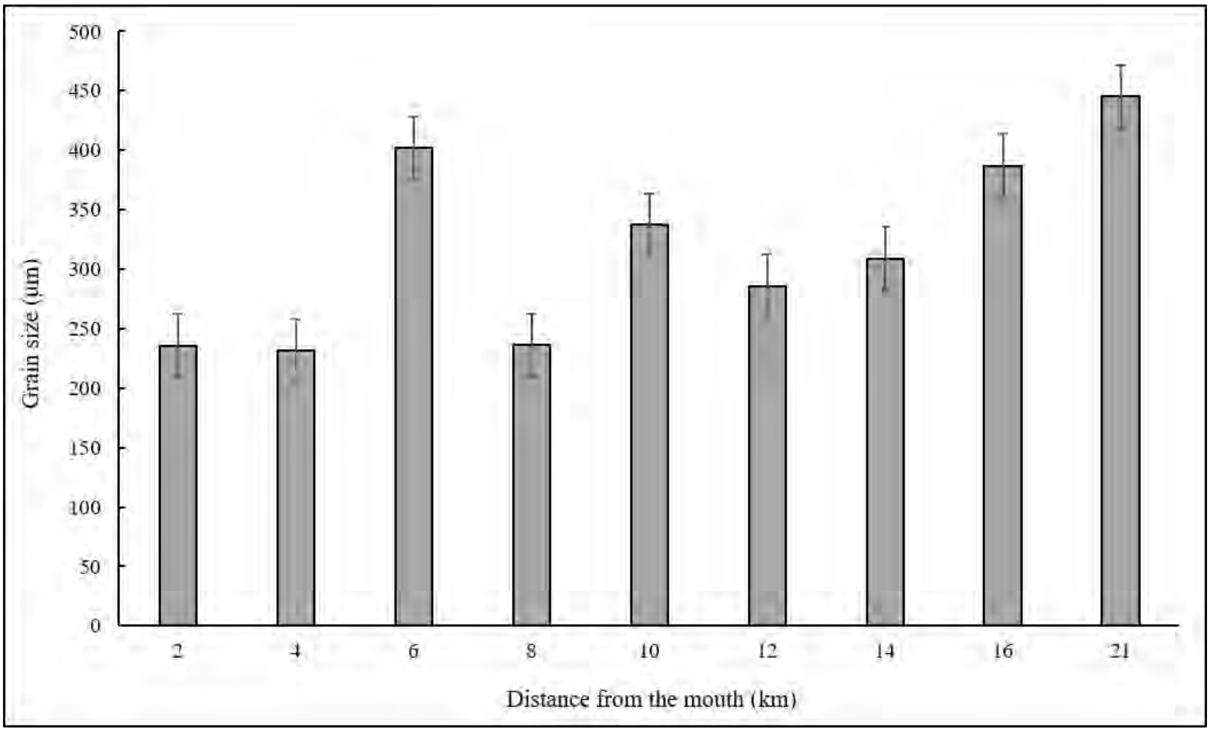


Figure 2.5: Percentage contribution of particle size of sediment collected in the Sundays Estuary

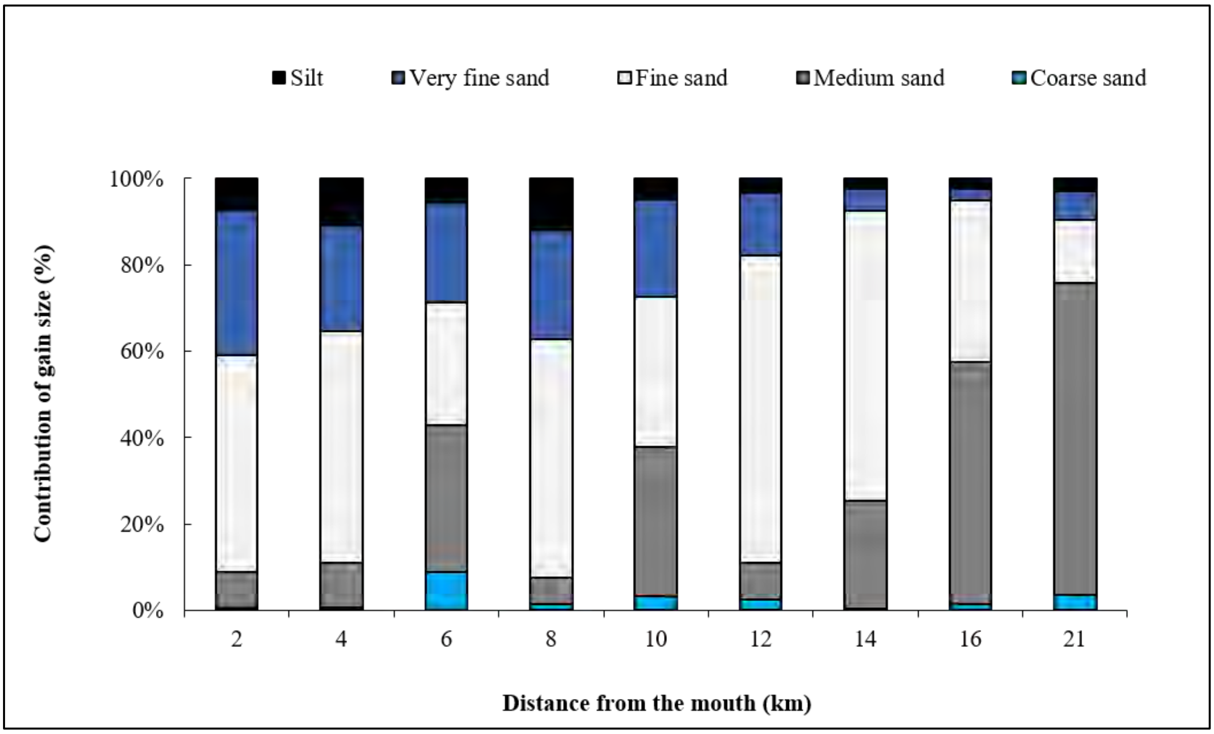


Figure 2.6: Percent contribution of grain size in different sites of the Sundays Estuary

Principal Component Analysis (PCA) in PRIMER v6 (Plymouth Routines in Multivariate Ecological Research) was conducted in order to visualize groupings of the sampling sites by estuarine reach. A draftsman plot was first generated to visually assess normality of mean bottom environmental variables per season. Environmental variables used in the analysis were temperature (°C), salinity (‰), sand (%), silt (%), turbidity (NTU), clay (%), organic content (%), pH and dissolved oxygen (mg/l). Organic content, pH and dissolved oxygen were removed from the analysis since they were correlated with other variables. Inter-correlations (Pearson's R) were performed on normalised environmental data. All variables were normalised and a PCA plot generated. An analysis of similarities (ANOSIM) was also undertaken to test for significant differences between reaches.

The first, second and third components of principal component analysis (PCA) explained 66.79%, 21.78% and 6.4% of the variation in environmental characteristics, respectively. Sampling sites were grouped significantly by reach (ANOSIM R = 0.472). Sites in the middle reaches were situated towards the middle of the ordination and characterized by intermediate environmental conditions (Figure 2.7). Sites in the lower reaches (Sites 2–3), together with the mouth (Site 1), were characterised by high clay, dissolved oxygen and salinity. Sites in the middle reaches (Sites 4–7) were characterized by intermediate conditions and showed greater seasonal variability. Sites in the upper reaches (Sites 8–9) were characterised by high sand content and low salinity (Figure 2.7).

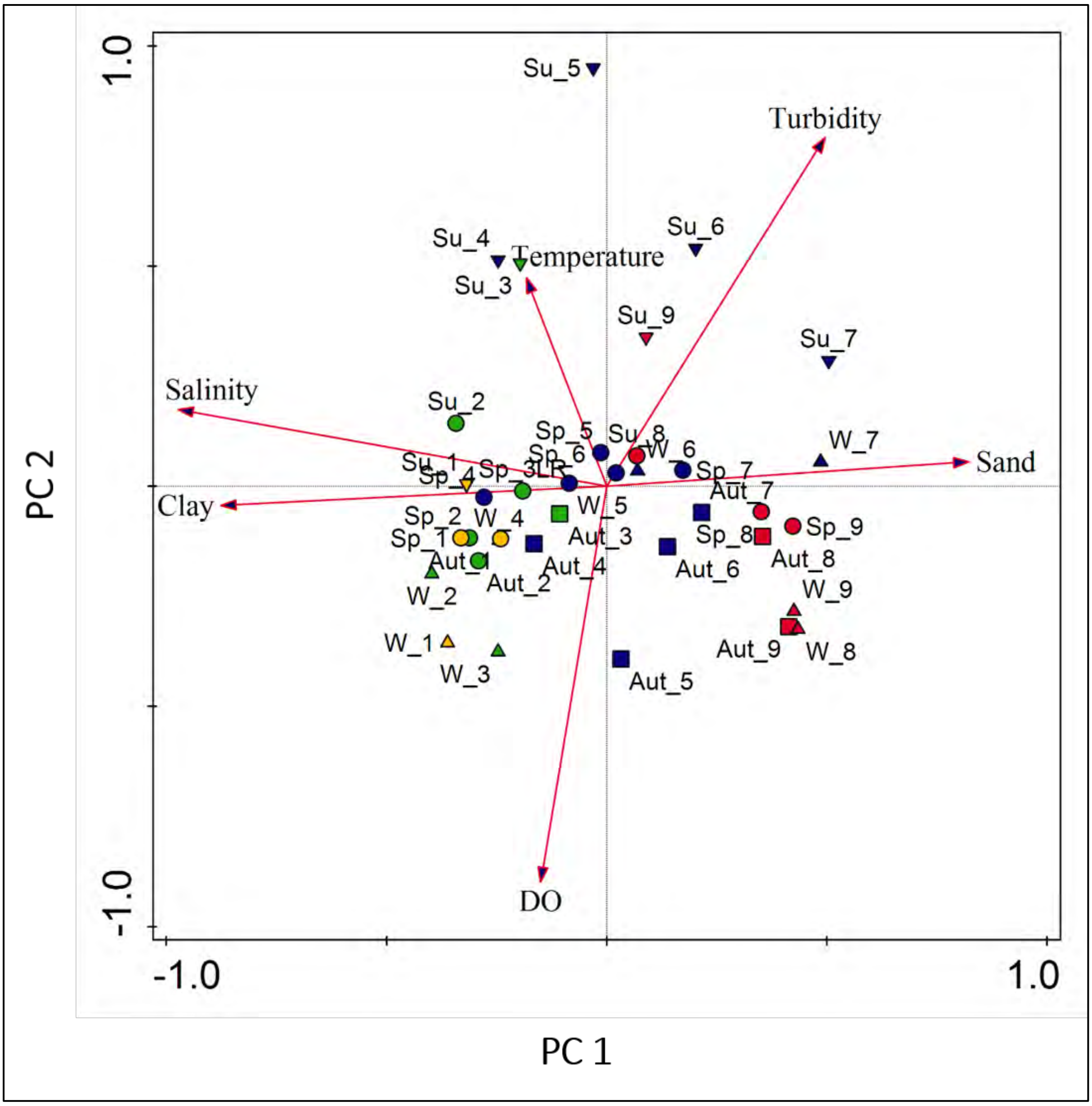


Figure 2.7: Principal component analysis (PCA) showing differences in sites by environmental variables along the length of Sundays Estuary (March 2018 to September 2019). Orange = mouth, green = lower reach, navy = middle reach, red = upper reach

Prey resources

Mesozooplankton

Mesozooplankton communities were sampled once-off (one replicate) during the day (28 August 2019) using an epibenthic sled at the nine sampling sites. The epibenthic sled was fitted with 200 µm mesh, with a semi-circular mouth with a radius of 18.5 cm. The net was mounted on a sled, so that the net was raised 7.5 cm above the sediment surface. The net was deployed from a boat in the channel of the estuary and towed for 35 m. After sampling, the sample was rinsed in a 1 L bottle and preserved with 4% formalin for analysis in the Ocean Science laboratory (Nelson Mandela University).

In the laboratory, samples were suspended in 2 L of distilled water and stirred so that the organisms remained in a homogeneous suspension. A 50 ml plastic jar was used for sub-sampling at midwater. As there was a low abundance of zooplankton in the samples, the whole 2 L was analysed in all samples. Zooplankton were identified to the lowest possible taxon and counted using Zeiss Stemi 508 dissecting microscope with magnification dependent on prey size.

Abundances were converted to densities (ind.m^{-3}) using the equation:

$$\text{Density (ind.m}^{-3}\text{)} = \frac{total\ abundance\ in\ sample}{sample\ volume}$$

To calculate the volume of filtered water (1.925 m^{-3}), the area of the sled mouth (0.055 m^{-2}) was multiplied by distance (35 m).

Mesozooplankton were composed of 14 taxa, and their composition varied along the length of the estuary (Table 2.3 and Figure 2.8). *Gastrosaccus brevifissura* (Mysid) and *Cypraea* sp. dominated the catches at the mouth (Site 1) and Site 3 in the lower reaches (Site 1 = 62.35% and 27.56% respectively; Site 3 = 58.26% and 38.81%, respectively). Ostracods comprised the third dominant component of the catch at these two sites (Site 1 = 9.94%; Site 3 = 2.93%). The copepod *Paracartia longipatella* completely dominated the catch at Site 2 in the lower reaches and comprised 94.91% of the catch. This site, although in the lower reaches, was consistently muddy (in contrast to the other lower reach sites). In the middle reaches (Sites 4 to 6) catches were dominated by the mysids *P. longipatella* (83.8%–89.5%) and *Mesopodopsis wooldridgei* (5.5%–16.2%). *Mesopodopsis wooldridgei* was abundant at Site 5 (64.65%) and Site 7 (71.64%) in the middle reaches, followed by mysid larvae (4.46 and 16.45%). Cladocera (24.80 and 57.65%), the copepod *Pseudodiaptomus hessei* (16.67 and 29.41%), chironomids (10.57 and 7.06%) and the decapod *Hymenosoma orbiculare* (34.15 and 2.35%) dominated catches in the upper reaches (Sites 8 and 9).

Table 2.3: Mean percent contribution of mesozooplankton taxa recorded in the Sundays Estuary in winter (28 August) 2019. Bold numbers represent dominant prey

	Distance from mouth (km)								
Mesozooplankton taxa	**2**	**4**	**6**	**8**	**10**	**12**	**14**	**16**	**21**
Mysids									
Mesopodopsis wooldridgei	0	0.25	0	**16.23**	**64.65**	5.51	**71.64**	0	0
Unknown Mysid larvae	0	0	0	0	4.56	0.03	**16.45**	0	0
Gastrosaccus brevifissura	**62.35**	3.4	**58.26**	0	0	2.26	0	0	0
Rhapalophthalmus terranatalis	0	0	0	0	1.04	0.14	2.27	0	0
Baetidae	0	0	0	0	0.07	0	0.19	1.22	3.53
Chironomidae	0	0	0	0	0	0.03	0.19	10.57	7.06
Decapoda									
Hymenosoma orbiculareraca	0	0	0	0	0.65	1.5	3.97	**34.15**	2.35
Gastropoda									
Cypraea	**27.56**	1.27	**38.81**	0	0	0	0	0	0
Cyclopoida									
Halicyclops	0.15	0	0	0	0	0	0	0	0
Unknown Cyclopida	0	0	0	0	0	0.85	0	0.81	0
Calanoida									
Paracartia longipatella	0	**94.91**	0	**83.77**	**28.52**	**89.5**	1.7	0.81	0
Pseudodiaptomus hessei	0	0	0	0	0.46	0	0.19	**16.67**	**29.41**
Ostracoda	9.94	0.17	2.93	0	0	0.17	3.02	10.98	0
Branchiopoda									
Chydorus	0	0	0	0	0.07	0	0.38	24.8	57.65

Mesozooplankton density varied along the length of the estuary, with the highest density (322.23 ± 58.83 ind.m^{-3}) recorded at the muddy site four km from the mouth (Site 2), with the copepod species *P. longipatella* contributing more than 90% of the catch at this site. The lowest mesozooplankton density (3.15 ± 8 ind.m^{-3}) was observed at the uppermost sampling site.

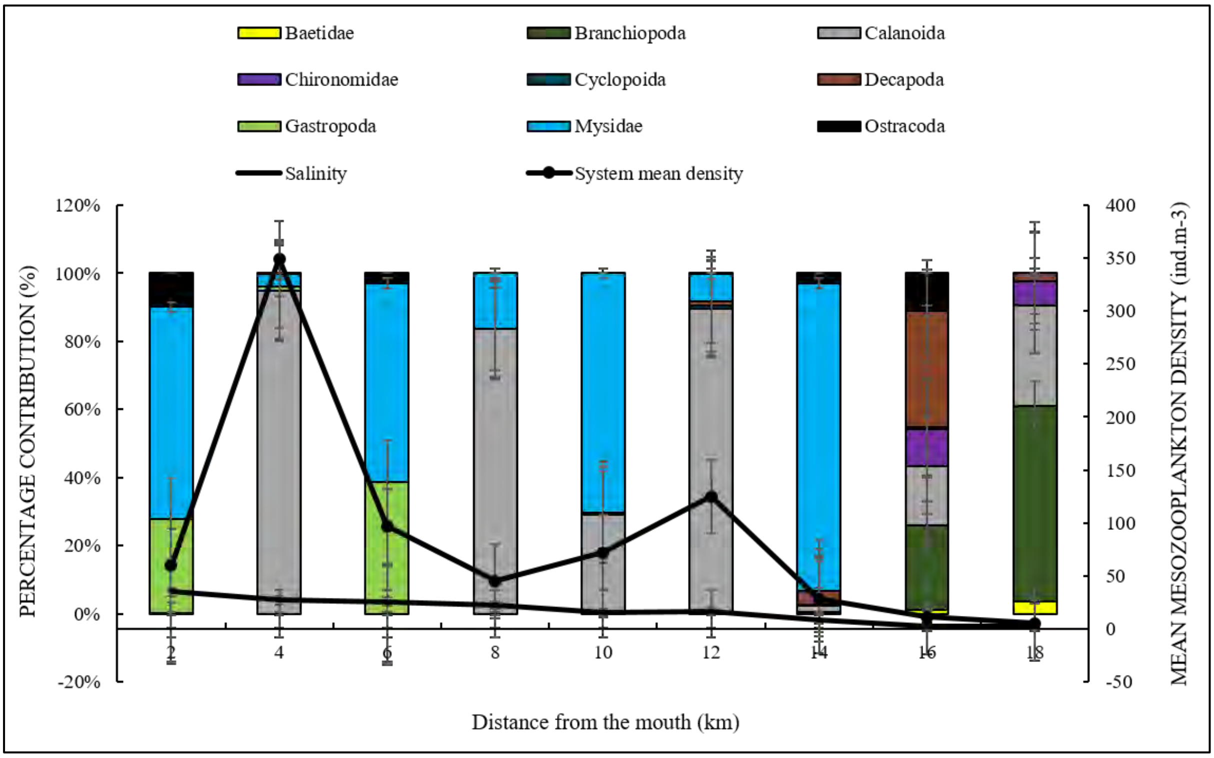


Figure 2.8: Mean percent contribution of dominant mesozooplankton taxa and total mesozooplankton density recorded in the Sundays Estuary in winter (28 August) 2019

Macrozoobenthos

Three replicate sediment samples were taken once-off at each of the nine sampling sites using a Van Veen grab (28 August 2019). The grab collected approximately 0.09 m^2 of sediment. Each sample was then rinsed through a 500 µm mesh sieve to collect the macrozoobenthos. Samples were then placed in a 1 L bottle with 7% magnesium chloride to anesthetize the animals. To rinse the magnesium chloride from the sample, a smaller size mesh (200 µm) was used. The samples were then later fixed in 4% formalin and stained using Rose Bengal. Sorting and processing was done under a Zeiss Stemi 508 dissecting microscope. Organisms were sorted based on their taxonomic groups (polychaetes, gastropods, bivalves) and then identified to the lowest possible taxon level and counted. The mean macrozoobenthic density was calculated as:

$$\text{Density (ind.m}^{-2}\text{)} = \frac{total\ abundance\ in\ sample}{grab\ area}$$

To obtain mean density, an average of the three samples was taken per sampling site.

A total of 24 macrozoobenthos taxa were recorded (Table 2.4 and Figure 2.9). The mouth (Site 1) and lower reach Site 3 (6 km from the mouth) had the highest diversity of macrozoobenthos, with 12 species recorded at these sites. Polychaete species (Spionidae and Capitellidae), unknown Ostracods, together with gastropods (Limacinidae and Tellinidae) dominated the species composition within these two sites (Polychaete comprising 23.58 and 15.57% respectively, Ostracoda: 6.13% and the gastropods comprised 23.58 and 22.64% of the catch). Two ostracods (Architectonicidae and Cypraeidae) dominated the catches at Sites 2 and 4 (Architectonicidae comprised 41.14, 74.38 and 56.58% respectively and Cypraeidae comprised 23.00, 23.05 and 6.76% respectively). Crab species (*Paratylodiplax* spp) were also recorded in high numbers at Site 2 in the lower reaches, comprising 14.56% of the catch. Polychaeta species (Spionidae spp.) were also recorded at Site 4 in the middle reaches (19.93%), with the contribution of polychaetes very high at Sites 5 and 6 in the middle reaches (comprising 69.09 and 74.59%). Polychaeta (Terebelida) dominated catches (55.40%) at Site 7. Chironomid species increased in abundance in the middle reaches (Site 4 2.85%, Site 5 3.64%, Site 6 13.26% and Site 7 19.18%). Ancylidae (gastropod) increased in abundance from sites 5–8, comprising 23.81% of the catch at Site 8. In the upper reaches (Sites 8 and 9), unknown Polychaeta, Cypridopina spp (gastropod), *Gandidiella japonica* (amphipod) and Gnathiidae spp (isopod) dominated the catches (unknown Polychaeta: 10.88 and 41.52%, Cypridopina spp: 15.65 and 22.56%, *Grandidiella japonica:* 4.76 and 9.03% and Gnathiidae spp: 19.05 and 2.35%).

Mean density varied along the length of the estuary (Figure 2.9), with the highest mean density observed in the lower reaches six km from the mouth (198.15 ± 745.22 ind.m^{-3}). The lowest value was observed in the middle reaches 10 km from the mouth (8.49 ± 28.76 ind.m^{-3}).

Table 2.4: Mean percent contribution of winter (28 August) 2019 macrozoobenthos taxa recorded in the Sundays Estuary, Eastern Cape, South Africa

	Distance from mouth (km)								
Macrozoobethos taxa	**2**	**4**	**6**	**8**	**10**	**12**	**14**	**16**	**21**
Amphipoda									
Grandidiella japonica	0.00	0.00	0.00	0.00	0.00	2.21	3.36	4.76	9.03
Isopoda									
Gnathiidae	0.00	0.00	0.00	0.00	0.00	0.00	0.24	**19.05**	2.35
Insecta									
Chironomidae	0.00	0.00	0.08	2.85	3.64	13.26	**19.18**	4.76	1.44
Calanoida									
Pseudodiaptomus hessei	0.47	0.21	0.00	0.00	1.82	0.00	0.00	0.00	0.00
Malacostraca									
Cumaceae	0.94	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Insecta									
Baetidae	0.00	0.00	0.00	0.00	0.00	0.55	0.24	0.68	0.00
Mysidae									
Rhopalophthalmus terranatalis	0.00	0.00	0.00	0.00	0.00	2.76	0.00	0.00	0.00
Mesopodopsis wooldridgei	0.00	0.00	0.08	0.00	1.82	0.00	0.00	0.00	0.18
Mysidae larvae	0.00	0.00	0.00	0.00	1.82	0.00	0.00	0.00	0.00
Decapoda									
Brachyura	0.94	0.00	0.16	0.00	0.00	0.00	0.00	0.00	0.00
Paratylodiplax spp	1.42	**14.56**	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Upogebia africana	0.00	0.00	0.08	0.00	0.00	0.00	0.00	0.00	0.00
Gastropoda									
Cypraeidae	2.36	**23.00**	**23.05**	6.76	0.00	0.00	0.00	0.00	0.00
Limacinidae	**23.58**	0.00	0.16	1.42	3.64	0.55	0.96	0.00	2.71
Architectonicidae	0.47	**41.14**	**74.38**	**56.58**	0.00	0.00	0.00	0.00	0.36
Ancylidae	0.00	0.00	0.08	0.36	5.45	2.21	8.63	**23.81**	**11.91**
Bivalvia									
Tellinidae	**22.64**	0.00	0.08	0.00	0.00	0.00	0.00	0.00	0.72
Cypridopina	0.00	0.84	0.00	0.00	0.00	0.00	0.00	**15.65**	**22.56**
Polychaeta	40.57	19.83	1.87	32.03	81.82	78.45	67.63	31.29	48.74
Spionidae	**23.58**	4.43	0.70	**19.93**	**69.09**	**74.59**	9.11	0.00	7.22
Nereididae	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.68	0.00
Capitellidae	**15.57**	5.70	0.47	7.47	**12.73**	0.55	3.12	6.80	0.00
Terebelida	1.42	0.00	0.70	4.63	0.00	3.31	**55.40**	**12.93**	0.00
Unkown polychaeta	0.00	9.70	0.00	0.00	0.00	0.00	0.00	**10.88**	**41.52**

Ostracoda	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

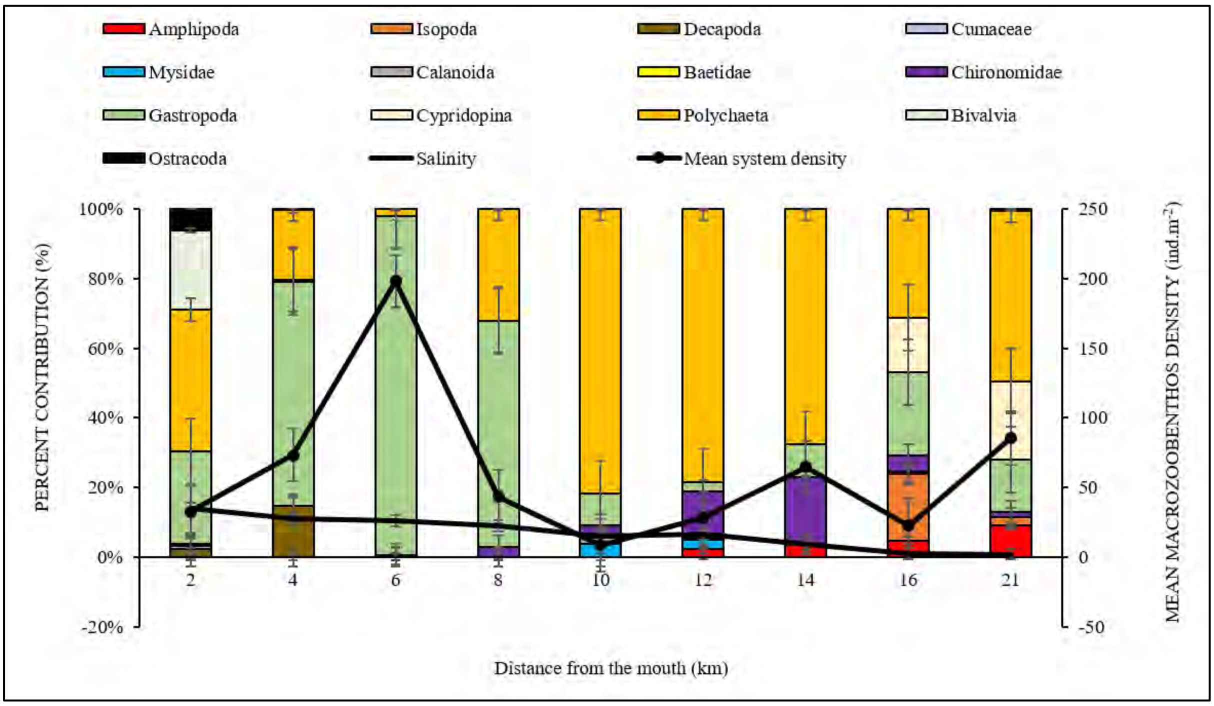


Figure 2.9: Mean percent contribution of winter (28 August) 2019 macrozoobenthos taxa recorded in the Sundays Estuary, Eastern Cape, South Africa

CHAPTER THREE

Reproductive biology of *Psammogobius knysnaensis* and *Caffrogobius gilchristi* in the Sundays Estuary

Introduction

Reproductive success determines the continued existence of species in a particular region. In addition, when species are sympatric and functionally analogous, they often compete for resources such as food and nesting sites. To avoid this situation, fish partition resources by size or species by inhabiting different habitats or feeding on different prey (Sala and Ballesteros, 1997). Therefore, the physiological characteristics of a habitat, space (nesting sites) and food availability (energy investment for reproduction) are the main drivers of reproductive partitioning of similar coexisting species, as they allow species to occur in different habitats, which minimizes their competition for nesting sites (Fitzgerald and Whoriskey, 1985; Persson, 1990).

When functionally similar, coexisting species compete for resources such as food and nesting sites in a particular environment, they tend to reproduce at different times or in different habitats to avoid behavioural interactions (Breitburg, 1987; Faria and Almada, 2001; Gertzen et al., 2016). This is due, in part, to aggressive species outcompeting other species for nesting sites, and this negatively affects the reproductive success of the less competent species (Breitburg, 1987). In the tidal pools west of Rivière-du-Loup Quebec, coexisting Three-spined stickleback *Gasterosteus aculeatus* (Linnaeus, 1758) and Blackspotted stickleback *Gasterosteus wheatlandi* (Putnam, 1867) both had low reproductive success, but the aggressive behaviour of *G. wheatlandi* enabled it to outcompete *G. aculeatus* for nesting sites (Fitzgerald and Whoriskey, 1985). In addition, when coexisting species reproduce at the same time, there is the possibility that the aggressive species may consume the eggs of the less competent species (Seehausen and Schluter, 2004). For example, because gobies potentially feed on fish eggs and fry, they could consume each other's eggs and fry when reproducing in the same habitats (Chotkowski and Marsden, 1999; Vašek et al., 2014). Gobies also have the potential to hybridize, and this was observed between the Dusky tripletooth goby *Tridentiger obscurus* (Temminck and Schlegel, 1845) and the Katsuyama *Tridentiger brevispinis* (Arai and Nakamura, 1972) in Lake Hinuma, Japan (Mukai et al., 2000).

Factors promoting the success of species in space-limited environments include their ability to reproduce throughout the year (Torricelli et al., 1985; Mofu, 2016); fast growth rates and short life spans enable them to mature early, resulting in high lifetime fecundity as well as per brood fecundity (Miller, 1961; Arruda et al., 1993; Azevedo and Simas, 2001; Mofu, 2016). This is evident within the Gobiidae family (Mofu, 2016). Assessing fish reproductive biology and relating it to abiotic and biotic factors can help to explain the variability seen in reproductive strategies among co-occurring species, and elucidate the effects of habitat selection which favour high reproductive success (Marua et al., 2003). Fecundity, which is defined as the number of eggs produced by fish (Healey, 2011), length at maturity and spawning period, can be used to estimate the abundance and reproductive success of fishes (Sarker et al., 2002). These biological attributes vary among fish species and with fish size (Sarker et al., 2002).

In order to observe whether different reproductive strategies promote coexistence, this chapter aimed to describe the reproductive biology of the Knysna sand goby *Psammogobius knysnaensis* (EM) and Prison goby *Caffrogobius gilchristi* (E & M) collected from the Sundays Estuary, as their reproduction has not been well described in South African estuaries (Bennett, 1989). Their reproductive period will explain how the species manage to coexist in the estuary and provide a better understanding of their high abundance in South African estuaries. The reproductive biology of the River goby *Glossogobius callidus* (E & F) has been studied in the freshwater population in the Sundays River (Mofu, 2016). Due to sampling constraints, as the upper reaches of the estuary are steep and not easy to seine net, and as individuals captured were mainly small individuals, the reproductive biology of this species could not be comprehensively studied in the estuary. Studying their reproductive biology was accomplished by determing (1) the spawning period, (2) length at maturity and (3) fecundity of *P. knysnaensis* and *C. gilchristi*. It was hyposythesised that *C. gilchristi* will start to reproduce at larger sizes and produce higher egg numbers, since individuals of this species are larger compared to the other species.

Materials and Methods

Sample collection

Sampling was conducted over a 12-month sampling period between September 2018 and August 2019. Sampling was conducted once a month using a small mesh seine net (30 m long x 2 m deep) with a 5 mm bar mesh. During sampling, the net was deployed in a semi-circle from the bank using a boat and then hauled to the bank by three to four people. One end of the net was held stationary during the net deployment. Seine netting was conducted until a minimum of 30 individuals per species were caught per month with sampling sites selected based on the possibility to pull the seine net. After capture, bycatch species were released back to the water alive and the study species were placed in a bucket containing water with 40 mg l^{-1} clove oil for humane euthanasia (Rhodes university ethical clearance number 2019-0543-750). The fish were stored in a cooler box containing ice for later laboratory analysis in Ocean Sciences (Nelson Mandela University).

Laboratory analysis

A total of 1090 individuals of *P. knysnaensis*, comprising 492 males and 598 females were caught, and a total of 949 individuals of *C. gilchristi*, comprising 497 males and 452 females were caught. In the laboratory, fish were first preserved in 5% formalin and later 70% ethanol. For analysis, all fish were measured for total length (mm TL), weighed (g), dissected and sexed. Males were characterised by a short and thin urogenital papilla while in females this was long and wide. Identifying the urogenital papilla was done under a Zeiss Stemi 508 dissecting microscope. The magnification size used was dependent on the specimen size. Gonadal developmental stages were classified following the procedure developed by Mofu (2016) for *G. callidus* in the Sundays River. Developmental stages are described in Table 3.1 for females and Table 3.2 for males. The weight (g) of the gonads together with the body mass with the gonads removed were recorded. After measuring, the gonads were then preserved in 70% ethanol.

Table 3.1: Gonad developmental stages for female *Glossogobius callidus* from Mofu (2016)

Stage	Macroscopic characteristics	Histological characteristics
Juvenile	Gonad appears as thin gelatinous band and hard to distinguish	Oogonia small compared to nucleus in a slightly basophilic cytoplasm
Resting	Sexes distinguishable, bigger than previous stage, but eggs not visible to naked eye	Early perinuclear oocytes and late perinuclear oocytes dominate the ovary
Developing	Ovary bigger than previous stage, occupying half the body size Whitish eggs visible	Primary yolk vesicle Cortical alveoli Zona radiata and zona granulosa
Ripe	Yellowish orange eggs clearly visible occupying most of the body cavity	Secondary yolk vesicle Hydrated oocytes with yolk globules

Spent	Ovaries appear deflated	Atretic oocytes

Table 3.2: Gonad developmental stages for male *Glossogobius callidus* from Mofu (2016)

Stage	Macroscopic characteristics	Histological characteristics
Juvenile	Gonad appears as thin gelatinous band and hard to distinguish	Empty lumen mainly surrounded by lobules containing spermatogonia
Resting	Testes distinguishable, bigger than previous stage,	Spermatocytes dominate lobules
Developing	Lobule thicker and testes enlarged greatly	Spermatocytes dominate Spermatids present in the lobules
Ripe	Testes larger and whitish in colour	Spermatozoa dominate the lumen
Spent	Testes appear deflated	Empty lumen containing residual spermatozoa

Spawning season

To determine the spawning season, visual assessment of the maturity stage of the gonads and monthly progression of the gonado-somatic index (GSI) were done. The gonado-somatic index was calculated by expressing the gonad mass as a percentage of the eviscerated body mass. High values are only observed during the spawning season. To determine the length of the spawning season, monthly GSI was calculated in Microsoft Excel only from individuals that were larger than mean length-at-maturity (L_m) using the equation:

$$GSI = \frac{gonad\ mass\ (g)}{Eviscerated\ body\ mass\ (g)} * 100 \qquad (1)$$

A chi-square contingency table (12 X 2) was used to determine the significant difference of monthly male and female GSI among species.

Length at maturity

The L_m is the length at which 50% of all individuals are mature (King 1995). This was calculated by fitting a logistic equation to the proportion of reproductively capable fish that were collected during the spawning season. The individuals capable of reproducing were those fish that had developed, ripe or spent gonads. The logistic function (fitted in Microsoft Excel) was expressed as (equation):

$$P(L) = \frac{1}{1 + e^{-(TL - Lm50)/\delta}} \qquad (2)$$

where *P(L)* is the percentage of fish that were found mature at the length TL, L_{m50} is the length at which 50% of the fish in the length class are mature, and δ is the steepness of the ogive (Weyl and Booth, 1999).

Curve fitting was performed using the Solver routine in Microsoft Excel using the least-squares method (Winker et al., 2010). Length at first maturity was taken as the length of the smallest mature male and female. Length at 100% maturity was the length at which all individuals in the sample were mature. The maximum likelihood estimates of the parameters were obtained by minimising the binomial negative log-likelihood of the form as illustrate in the equation below:

$$-LL = \sum_i \quad [miln\,(\hat{P}) + (ni - mi)in(1 - \hat{P}i)] \qquad (3)$$

Where $\hat{P}i$ is the proportion predicted of mature fish in length class i, ni is the number of individuals sampled and mi is the number of these individuals that are mature. Likelihood ratio tests were used to test the null hypothesis that L_{m50} values were equal between sexes. Individuals staged as developing, ripe and spent were used to calculate length at maturity.

Fecundity

A total of 47 and 50 individuals were analysed to calculate relative fecundity for *P. knysnaensis* and *C. gilchristi*, respectively. A Zeiss Stemi 508 dissecting microscope was used for hand counting of eggs in order to determine fecundity (relative fecundity), which is the number of mature, yolked and unovulated eggs of fish before spawning (Indira et al., 2013). Fish were collected during the period of high reproductive activity (December 2018 – February 2019). To determine relative fecundity, the number of all vitellogenic oocytes were divided by the fish body mass.

Results

Female *P. knysnaensis* ranged from 18–62.19 mm total length (TL) and males ranged from 17.06–69.40 mm TL. For *C. gilchristi*, females ranged from 22.58–69.28 mm and males ranged from 17.26–94.00 mm TL. Distinguishing between sexes in individuals below 37.00 mm TL in *C. gilchristi* was difficult, as all appeared to have male characteristics but it was easy to differentiate in individuals above this size.

Spawning season

Monthly changes in gonadosomatic index (GSI) for both species are shown in Figure 3.1. In male and female *P. knysnaensis* mean GSI did not exceed 2% and 8%, respectively, meaning that this species does not have big gonads (Figure 3.1a). The mean GSI of males and females was highest in austral summer, reaching its peak in January (Figure 3.1a). For both genders, a sharp decrease was observed in autumn (March, April, May) (Figure 3.1a).

Male and female GSI of *C. gilchristi* did not exceed 2% and 14% respectively (Figure 3.1b). In both sexes GSI was highest in austral summer (November to February) and austral winter (June to September), with peak spawning occurring in October, January and August (Figure 3.1b) meaning that there was overlap in peak spawning of the two species. The lowest GSI was also observed in autumn. When comparing both species, there was no significant difference in male *P. knysnaensis* and *C. gilchristi* ($p > 0.05$, $X^2 = 0.25$, df = 11) and female *P. knysnaensis* and *C. gilchristi* ($p > 0.05$, $X^2 = 4.82$, df = 11) GSI.

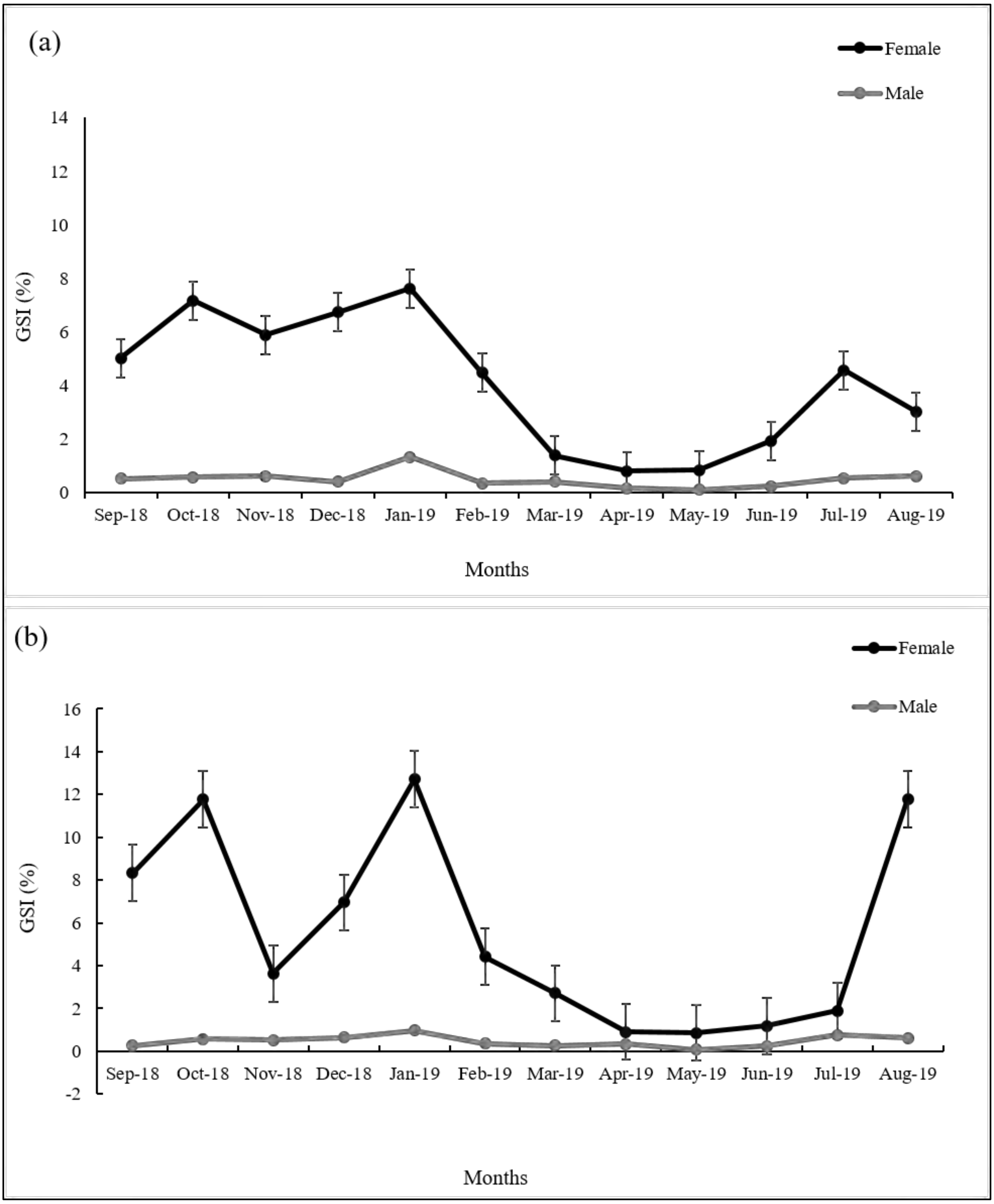


Figure 3.1: Average Gonadosomatic index of male and female *Psammogobius knysnaensis* (a) and *Caffrogobius gilchristi* (b) from the Sundays Estuary from September 2018–August 2019

Length at maturity (L_m)

The smallest mature male and female *P. knysnaensis* were 29.82 and 26.68 mm TL, respectively (Figure 3.2a, b). Male *P. knysnaensis* reached 50% maturity at 45.00 mm TL, while in females it was observed at 40.00 mm TL. Similarly, female and male *C. gilchristi* together with female *P. knysnaensis* reached 100% maturity at 60 mm TL. Male *P. knysnaensis* reached 100% maturity at 50.00 mm TL.

The smallest mature male *C. gilchristi* was 31.62 mm TL, with 38.00 mm TL for the females (Figure 3.2c, d). Both male and female *C. gilchristi* reached 50% maturity at 45.00 mm TL. All males and females larger than 60.00 mm TL were mature. There was no significant difference in male and female 50% maturity between the two species ($p > 0.05$).

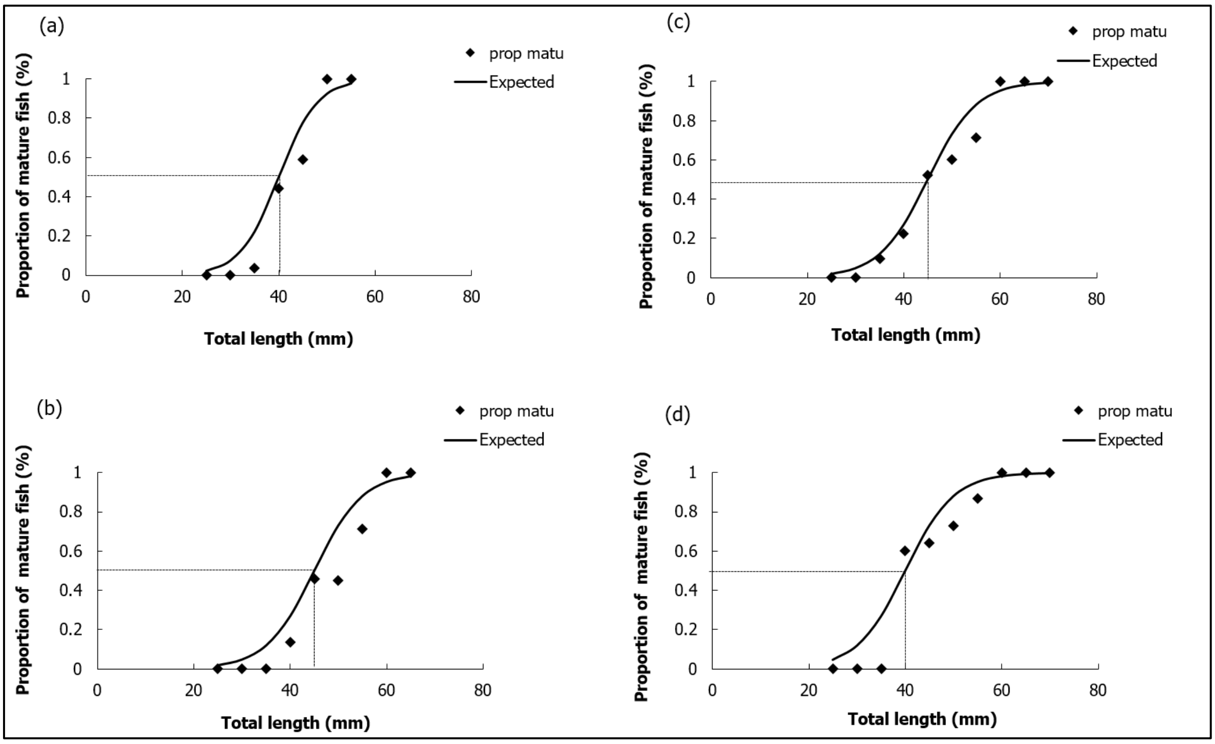


Figure 3.2: Length at 50% maturity of female (a) and male (b) *Psammogobius knysnaensis* together with male (c) and females (d) *Caffrogobius gilchristi* collected from the Sundays Estuary (December 2018–February 2019)

Fecundity

For *P. knysnaensis* there was no increase in the number of eggs before spawning with fish size ($R^2 = 0.0037$) and for *C. gilchristi* the number of eggs before spawning increased ($R^2 = 0.2475$) with fish size (Figure 3.3a, b). *Psammogobius knysnaensis* (maximum TL = 62.19 mm) had higher fecundity than *C. gilchristi* (Maximum TL = 69.28 mm). *Psammogobius knysnaensis* had higher absolute fecundity (2321.6), regardless of *C. gilchristi* (1650.17) having a stronger fecundity/length relationship. *Glossogobius callidus* had the lowest fecundity (350) (Mofu, 2016). A summary of the reproductive strategy all three species is provided in Table 3.3.

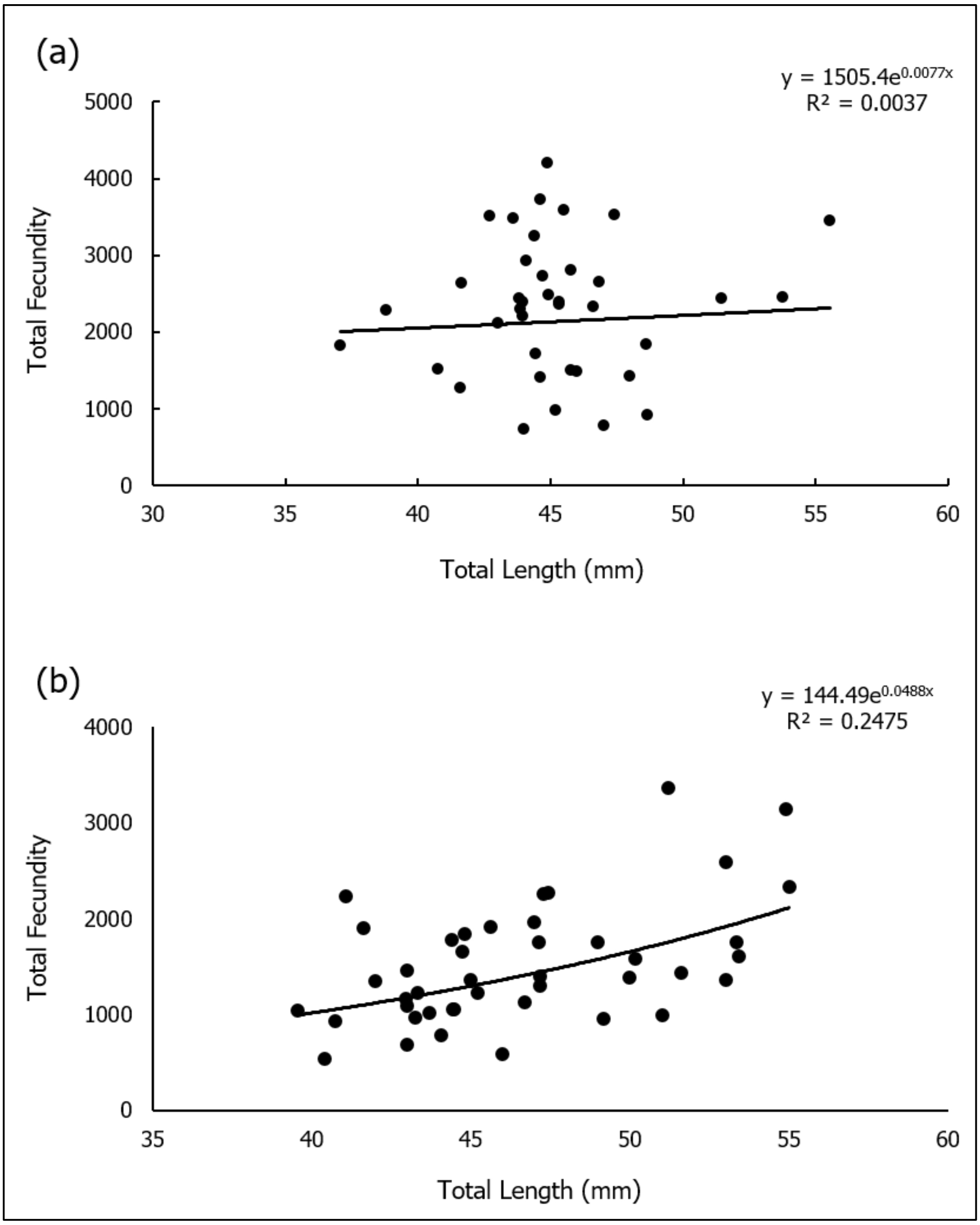


Figure 3.3: Total fecundity of *Psammogobius knysnaensis* (a) and *Caffrogobius gilchristi* (b) sampled from the Sundays Estuary (December 2018–February 2019)

Table 3.3: Life history strategies of *Psammogobius knysnaensis, Cafrogobius gilchristi* and *Glossogobius callidus* in the Sundays Estuary.

	Caffrogobius gilchristi	***Glossogobius callidus***	***Psammogobius knysnaensis***
Category (Whitfield 2019)	Ib (breeds mainly in estuaries also marine)	I (breeds mainly in estuaries also freshwater)	I (breeds mainly in estuaries also marine)
Spawning season	Spring and summer (this study)	Spring and summer (Mofu 2016)	Spring and summer (this study)
LM50	45 mm TL both male and female (this study)	35 mm (Boullé 1989)	Male 45 mm TL Female 40 mm TL (this study)
Fecundity	1650.168 (this study)	350 (Boullé 1989)	2321.6 (this study)

Discussion

Determining species-specific differences in coexisting species reproductive strategies may further our understanding of their mechanisms of coexistence. The three species had overlapping spawning seasons, with *P. knysnaensis* and *C. gilchristi* reaching peak spawning at the same time (January) while that of *G. callidus* in the Sundays River is known to be in December (Mofu, 2016). *Psammogobius knysnaensis* seemed to have higher fecundity than *C. gilchristi* and *G. callidus*. It had higher GSI than *C. gilchristi,* meaning that it requires more food during the spawning season, because reproduction uses energy (Santos et al., 2010). All species started maturing at similar sizes (Boullé (1989) estimated that *G. callidus* matures at 35 mm).

Reproduction of all species seemed to occur in the warm months (spring and summer), with peak reproduction observed in summer. This period coincides with high food availability, as phytoplankton and zooplankton abundance are highest in estuaries during this period (e.g., Froneman, 2001). During periods of high prey availability there is less competition for food, allowing for higher energy investment in reproduction. In this estuary, low zooplankton abundance have been recorded in winter as observed by Jerling and Wooldridge (1995) and there was less reproduction during this period, as reproduction requires energy (Santos et al., 2010). Multiple spawning and the high food availability in summer is favourable for *P. knysnaensis. Psammogobius knysnaensis* is characterised by high fecundity, which was not related to fish size, while *C. gilchristi* had lower fecundity, which increased with fish size. These results suggest that *P. knysnaensis* uses more energy during spawning than *C. gilchristi* and *G. callidus,* which have lower fecundity, since energy is used during reproduction (Santos et al. 2010).

Fish species with a short life span invest highly in reproduction. In a study by Grabowska and Przybylski (2015) in Europe, small-sized, short-lived fish species within the Cyprinidae, Ictaluridae, Gasterosteidae, Cottidae, Centrarchidae, Odontobutidae and Gobiidae families characteristically had early maturity, extended spawning seasons and were able to spawn multiple times. This was also evident in this study, as both species had an extended spawning season. This extended spawning season is common in gobies (Koutrakis and Tsikliras, 2009; Dinh, 2018). Although multiple spawning was not investigated in this study, gobies are known to be multiple spawners and this enhances reproductive fitness and success, as estuaries are dynamic environments (Nicholson et al., 2008). In the Venice Lagoon, marbled goby, *Pomatoschistus marmoratus* (Risso, 1810) was observed to be a multiple spawner (Mazzoldi and Rasotto, 2001). *Pomatoschistus marmoratus* fecundity also increased with fish size. Their short life span, which can be less than a year for some species, encourages their high recruitment success (Arruda et al., 1993; Huo et al., 2014). Other species such as the Padanian goby *Padogobius martensi* (Bonaparte, 1846) and burrowing goby *Trypauchen vagina* (Bloch and Schneider, 1801) can also spawn more than once in the same spawning period (Cinquetti and Rinaldi, 1987; Dinh, 2018).

All study species mature early, which promotes high recruitment as they have a short life span. Because all species start to reproduce at less than 40.00 mm TL this means that reproduction starts in their first year. The fast growth rate in gobies enables them to reproduce within the first year (Bouchereau and Guelorget, 1997; Azevedo and Simas, 2000). In the Mediterranean striped goby, *Gobius vittatus* (Vinciguerra, 1883) reproduction also commences at less than 40.00 mm TL (Kovačić, 2007b). Another goby species, *P. marmoratus* also starts to reproduce at less than 40.00 mm (Koutrakis and Tsikliras, 2009). This could be linked to the fact that gobies have a short life span (Arruda et al., 1993). Rock goby *Gobius paganellus* (Linnaeus, 1758) and *G. vittatus* also reproduce within the first year (Azevedo and Simas, 2000; Iida et al., 2011). This could explain the early reproduction observed in the two study species. The high population persistance of gobies in estuaries is promoted by their high reproductive rates (high number of eggs), with some species capable of reproducing twice a year (MacInnis and Corkum, 2000; Koutrakis and Tsikliras, 2009; Chapter 3). Their fast growth rate, which enables them to reproduce within their first year, also promotes their high abundance (MacInnis and Corkum, 2000).

Similar coexisting fish species compete for nesting sites, and this was observed in coexisting *G. aculeatus* and *G. wheatlandi* (Fitzgerald and Whoriskey, 1985). This competition between coexisting fish species can negatively suppress their reproduction, more specifically if they reproduce at the same size (Thresher, 1983; Persson, 1990). As *P. knysnaensis* and *C. gilchristi* mature at the same size they have a high potential to compete for nesting sites and consume each other's eggs. All species use the underside of rocks and other hard surfaces to attach their eggs (Wasserman et al., 2015; Wasserman et al., 2017; Whitfield, 2019). Competition for food can also suppress the reproductive success of fish, as they depend on energy investment for reproduction (Fraser and Gilliam, 1992; Santos et al., 2010).

Conclusion

The three species reproduced during the same period (austral summer), with peak reproduction occurring in January. *Psammogobius knysnaensis* egg production was higher than the other two species and was not affected by size, whereas *C. gilchristi* and *G. callidus* produced fewer eggs (Mofu, 2016). All species started to reproduce at sizes less than 40.00 mm, suggesting that reproduction starts in their first year. As the reproductive period of the three species overlaps, this suggests that they need to partition resources (food and space), particularly during peak spawning. In contrast to the initial hypothesis, *P. knysnaensis* showed higher fecundity than the other species whereas *Caffrogobius gilchristi* started to reproduce at larger sizes as hypothesised.

CHAPTER FOUR

Interspecific differences in the feeding morphology of *Psammogobius knysnaensis, Caffrogobius gilchristi* and *Glossogobius callidus* from the Sundays Estuary

Introduction

An organism's morphology may have an impact on their feeding behaviour, which mayimpact their ecological functioning (Clifton and Motta, 1998). As such, the survival and reproductive success of fishes is influenced by food limitation. For example, when there are not enough food resources, mortality, slow growth (Houde, 1974; Bagarinao, 1986; Silva et al., 2007) and delayed reproduction occurs (Cerdá et al., 1994). The morphology (or body constructions) of fishes dictates their feeding, since it influences their ability to locate, chase, capture and ingest prey (Sibbing and Nagelkerke, 2001). The feeding process in fish involves different processes such as prey location, chasing, capturing, mastication and digestion (Sibbing and Nagelkerke, 2001). Transportation describes the process in between capturing and mastication. Transportation also occurs after chewing, whereby the food particle is transported to the gut (Sibbing and Nagelkerke, 2001). There are various morphological structures involved in this process.

Feeding morphological structures perform different functions during prey capture and processing, with the first stages involving prey location and chasing. To locate prey, fishes use their eyes in clear water and their taste buds in unclear turbid water (Al-Hussaini, 1949; van der Meer and Anker, 1984; Fernald, 1988). After prey has been located fish will reduce their distance from the prey by means of chasing or ambush (van Leeuwen and Muller, 1984). This is facilitated by a streamlined fusiform body of about equal width and depth (Webb, 1984; 1988). This, together with the narrowing of the caudal fin and small anal fin area, reduces water drag and promotes high chasing speed (Webb, 1984; 1988). When the distance from the prey has been reduced by chasing prey, capturing occurs, and this involves different morphological structures. For food intake, the oral gape and pharyngeal gape limit and determine the size of prey entering the mouth (size selection) (Stoner and Livingston, 1984; Wainwright and Richard, 1995; Mittelbach and Persson, 1998). Gill rakers act as sieves by determining the size of prey that can be retained (van den Berg et al., 1993). Then the postlingual organ width facilitates transport of the food to the chewing cavity (Sibbing et al., 1986). Once the food has been transported to the chewing cavity, mastication begins and later, transportation to the gut occurs. Pharyngeal teeth are involved in this process, and determine the amount of deformation after chewing (Sibbing, 1991), as well as the type of prey that can be consumed (Al-Hussaini, 1949; Stoner and Livingston, 1984). After mastication, the food is transported to the gut for digestion, with longer guts resulting in higher digestion time than smaller guts (Verigina, 1991).

Feeding morphological traits between trophic dimensions (grouping of fish species by diet) and different size individuals of the same species may limit the range of phenotypes that can be consumed (Wainwright and Richard, 1995), as the ability of predators to capture and consume prey has an underlying functional basis and is restricted by their various morphological features (Wainwright, 1988). These feeding morphological traits change during fish developmental stages and trophic dimensions resulting in changes in feeding performance (Al-Hussaini, 1949; Stoner and Livingston, 1984; Clifton and Motta, 1998; Kabasakal, 2001; Sibbing and Nagelkerke, 2001; Collar et al., 2009). For example, Werner (1977) determined that large-mouthed fishes are more efficient at feeding on fish than small-mouthed fishes. Apart from the differences caused by size and trophic dimension, the type of prey exposure may also affect feeding morphological traits (Svanbäck and Eklöv, 2002).

Populations of the same species in different environments may have different feeding morphological traits in response to resource availability in their feeding environments (Mittelbach et al., 1999; 1992; Svanbäck and Eklöv, 2002). This may be the result of local adaptation to the type of prey available (Mittelbach et al., 1999; 1992). For example, if the feeding environment is dominated by hard prey such as gastropods, fishes may develop hard jaw muscles (Mittelbach et al., 1999; Gerry et al., 2013) as they require strong muscles during mastication (Osenberg et al., 1992). In a study by Clifton and Motta (1998), individuals whose diet was dominated by hard prey had stronger pharyngeal jaw musculature than individuals from the same species feeding on soft prey. Pharyngeal jaw musculature is related to body size, with large body sizes resulting in stronger pharyngeal jaw musculature than small sized fish, as small fishes have smaller muscles (Clifton and Motta, 1998). Another study by Gerry et al. (2013) found that individuals of bluegill *Lepomis macrochirus* inhabiting an area with high numbers of gastropods in Lake Waban (USA) had larger pharyngeal jaws than individuals collected in an area with low numbers of gastropods, even though the study was conducted within the same lake.

As feeding morphological traits differ between size classes of the same species and trophic dimensions, their level of change can be used as a valuable tool for estimating the level of food competition between coexisting species (Clifton et al., 1998; Kumar et al., 2017). Differences in feeding morphological traits between closely related coexisting species may promote coexistence, and prevent the local exclusion of the less competent species (Kumar et al., 2017). The 'Food-Fish model' developed by Sibbing and Nagelkerke (2001) links feeding morphological traits and the ability of fish species to utilise different food resources. This model can be used to determine the trophic profile and mode of feeding in closely related fish species (Sibbing and Nagelkerke, 2001; Nagelkerke et al., 2018). In this model, differences in the trophic morphology of species are used to predict their potential to feed on different diets (Wainwright and Richard, 1995). Conversely, if the trophic profiles of different species overlap, this indicates the potential for similar diets (Nagelkerke et al., 2018).

In this chapter, inter-specific differences in the feeding morphology of *Psammogobius knysnaensis, Caffrogobius gilchristi* and *Glossogobius callidus* were explored in order to predict dietary differences and similarities between the three co-occurring species. The species were selected since they are closely related and are assumed to have high similarities in diet (Sibbing and Nagelkerke, 2001). It was hypothesised that *P. knysnaensis* and *C. gilchristi* would be morphologically different from each other, as they regularly co-occur in the lower reaches of estuaries (after Nodo et al., 2017; 2018).

This was accomplished by determining the following for the three species:

- Overall trophic morphology of each species
- Their capacity to feed on different aquatic food types using the Food-Fish model (FFM)

Materials and methods

Fish sampling

A total of 54, 60 and 48 individuals were collected for *P. knysnaensis, C. gilchristi* and *G. callidus*, respectively, from the Sundays Estuary in July 2018. Sampling sites were selected based on the possibility to conduct seine netting. Sampling was conducted using a small mesh seine net (30 m x 2 m) with a 5 mm bar mesh. During sampling, the net was deployed in a semicircle from the bank using a boat and then hauled to the bank by three or four people. One end of the net was held stationary during the net deployment. After capture, bycatch species were released back to the water alive and the study species were placed in a bucket containing water with 40 mg l^{-1} clove oil for humane euthanasia (Rhodes university ethical clearance number 2019-0543-750). The fish were then kept in a cooler box containing ice for later laboratory analysis in Ocean Science (Nelson Mandela University).

Laboratory analysis

In the laboratory, a total of 34 feeding traits were measured in each individual fish using digital calipers, and a Zeiss Stemi 508 microscope was used for measurements of less than 2 mm. Measurements followed those detailed in Nagelkerke et al. (2018) and Sibbing and Nagelkerke (2001) for cyprinids. Functional morphological traits measured are shown in Table 4.1 and illustrated in Figure 4.1. The presence or absence of oral teeth, gill raker secondary profiling and pharyngeal papilliform teeth were represented by 0/1, with 0 meaning absent and 1 representing present.

Table 4.1: Feeding morphological traits measured on *Psammogobius knysnaensis, Caffrogobius gilchristi* and *Glossogobius callidus* collected from the Sundays Estuary in July 2018, modified from Sibbing and Nagelkerke (2001); and Nagelkerke et al., The most essential adaptation required for fishes to occur in estuaries is the ability to adjust to changing salinity regimes (Whitfield, 2015; 2018). Numbers represent traits labels in Figure 4.1. Descriptions of the traits measured are only provided for those not clearly illustrated in Figure 4.1.

	Morphological trait	**Abbreviation**	**Description**	**Unit**	**Figure 4.1**
External body measurements	Body mass	BM		g	
	Total length	TL		mm	2
	Standard length	SL		mm	1
	Head length	HL	Excluding membrane of operculum	mm	9
	Body depth	BD		mm	3
	Body width	BW		mm	4
	Caudal peduncle depth	CPD		mm	5
	Anal fin length	AFiL		mm	6
	Anal Fin Base length	AfiBL		mm	7
External head measurements	Barbel presence	Ba		0/1	
	Oral teeth presence	TOT		0/1	

	Oral gape width	GW	Internal width oral gape	mm	13
	Oral gape height	GH	Internal height oral gape	mm	17
	Oral gape axis	OGAx	Ranging from terminal to sub- or supra-terminal; measured as 90° - OGAx	Degrees	12
	Eye diameter	ED		mm	15
	Snout length with mouth closed	Prot_cl		mm	10
	Snout length with mouth opened	Prot_op		mm	16
	Lower jaw length	LJL	From anterior tip to its posterior joint	mm	11
	Postorbital length	POrL		mm	14
	Operculum depth	OpD	From skull to point where sub-operculum and inter-operculum meet	mm	8
Internal measurements (after decapitation)	Gut length	GuL	Between pharyngeal jaws and anus	mm	
Internal measurements (dissection of jaw apparatus)	Hyoid length	HyL	Length hyoid bar (ceratohyal and hypohyal bones)	mm	
	Lower jaw-suspensorium length	LJSL	Length hyoid bar / length lower jaw – suspensorium bar	mm	
	Input closing lever of the lower jaw	LJin	Input-lever lower jaw for closing the mouth	mm	
	Output closing lever of the lower jaw	Ljout	Output-lever lower jaw for opening or closing the mouth	mm	
Internal measurements (dissection of branchial basket)	Gill raker length	GiRL	Average length of 10 rakers, lateral on arch 2	mm	21
	Gill raker distance	GiRD	Average of 10 inter-raker distances, lateral on arch 2	mm	20
	Gill raker secondary profile	GiRPr	Raker outgrowths, scaled from absent (1) to very elaborate (5)	05-Jan	
	Postlingual organ width	PLOW	Width of the oral floor between the left and right second gill-arch	mm	18

Internal measurements (dissection of pharyngeal jaws)	Pharyngeal papilliform teeth presence	TPT1		0/1	
	Pharyngeal molariform teeth presence	TPT2		0/1	
	Interdigitation of pharyngeal teeth	PJInt		0/1	
	Pharyngeal jaw symphysis length	PJSymL	Length of the symphysis between left and right pharyngeal jaw	mm	19
	Pharyngeal jaw mass	PJM	Wet mass of cleaned pharyngeal jaw (average of left and right)	g	

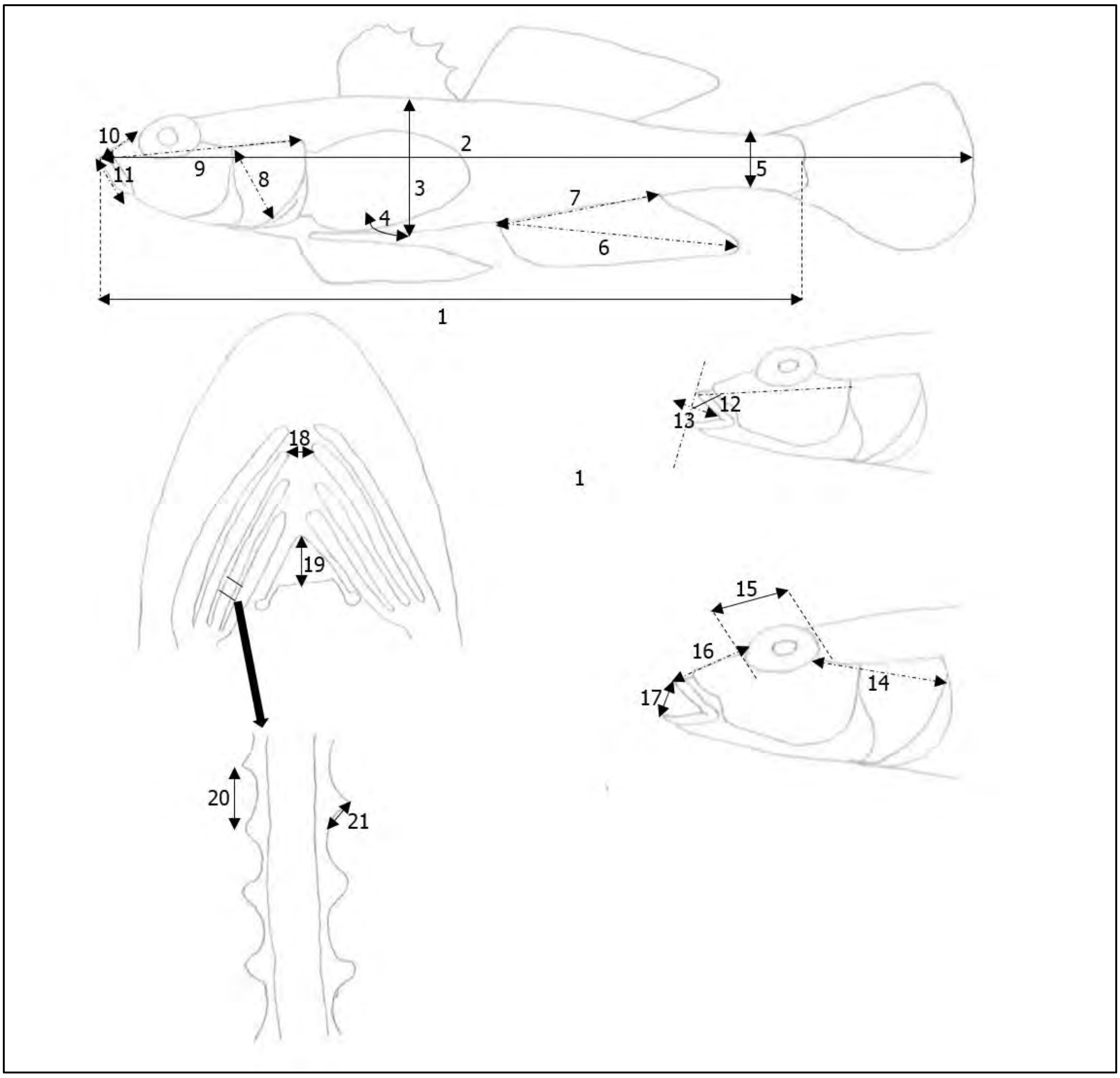


Figure 4.1: Illustration of the morphological traits measured on *Psammogobius knysnaensis, Caffrogobius gilchristi* and *Glossogobius callidus* collected from the Sundays Estuary in July 2018, using *P. knysnaensis* as an example. Internal head measurements are not illustrated as their accurate drawing requires an X-ray scanning of the fish head. Different numbers represent measured traits with descriptions provided in Table 4.1

Data analysis

All data analysis was conducted in R statistical software (3.5.1) using the procedure outlined in Sibbing and Nagelkerke (2001). Metric variables were expressed as a proportion of standard length (SL) and data were then standardized by subtracting the mean value (per variable) and dividing by the standard deviation. Each measured morphological trait was first plotted against the standard length of the fish to check for outliers. Correlations of variables were constructed for each species to exclude variables that were not well measured, or to avoid collinearity. A Principal component analysis (PCA) was then performed on the standardised data to visualise the overall trophic morphology of each fish species using R statistical software (3.5.1). The distance of the species to others represents the level of similarity in the trophic predictions. Species that are close to each other and species that are overlapping in the plot potentially consume the same food types, compared to the ones that are further from each other.

The potential food niches of each species were then predicted by comparing the standardised morphological traits measured for each species and within species, against values derived for these same traits for food specialists for the Food-Fish model (FFM) (Table 4.2; Sibbing and Nagelkerke, 2001). Sibbing and Nagelkerke et al. (2001) determined the relationship between morphological traits and the ability of food specialists (cyprinid fish) to eat different food types (phytoplankton, sessile algae, macrophytes, seeds, detritus, micro-crustaceans, macro-crustaceans, larvae/worms, macro-insects, molluscs and fish). This relationship is expressed as positive and negative values ranging between -2 and +2, with +2 indicating very high demand, +1 high demand, 0 average demand, -1 low demand and -2 very low demand (Table 4.2). This matrix of values (morphological trait versus food type) is referred to as the food specialist profile (FSP). A Kendall’s tau correlation was used to correlate FSP values with the morphological measurements of each individual fish. The outcome, called the trophic profile (TP), represents the capacity of each individual fish to utilise each of the tested food types in the wild (Sibbing and Nagelkerke et al., 2001).

Table 4.2: Demands on relative size and shape of elements comprising the feeding system to enhance feeding on different food types. 2 = very high demand, 1 = high demand, 0 = average demand, -1 = low demand and -2 = very low demand (After Sibbing and Nagelkerke 2001).

Food specialist	**Body depth**	**Caudal peduncle depth**	**Head length**	**Eye diameter**	**Protrusion length**	**Gut length**	**Lower jaw length**	**Gill raker length**	**Gill raker distance**	**Postlingual organ width**	**Lower jaw closing force efficiency**	**Volume capacity operculum**	**Operculum area**	**Gill arch resistance**	**Relative gape area**	**Velocity suction**	**Hyoid length**	**Gape size**	**Oral gape axis**	**Oral teeth presence**	**Pharyngeal molariform teeth**
Phytoplankton townet	-1	-1	1	0	0	1	0	2	-2	0	0	0	0	2	1	0	0	2	-1	0	1
Phytoplankzon pump	0	0	1	0	0	1	0	2	-2	0	0	1	1	2	0	0	0	1	-1	0	1
Algae	0	0	-1	0	0	1	-1	1	-1	0	1	0	0	0	0	0	0	0	0	0	1
Plants	0	0	-1	0	-2	0.5	-2	0	0	1	2	0	0	0	0	0	0	0	0	1	1
Seeds	1	0	0	0	1	0	0	0	0	0.5	0	0	0	0	0	0	0	0	0	1	1
Detritus	1	0	0	0	2	2	0	1	-1	0	0	0	0	0	0	0	0	-1	1	1	0
Microcrustaceans townet	-1	-1	1	0	0	0	0	2	0	0	0	0	0	2	1	0	0	2	-1	0	1
Microcrustaceans pump	0	0	1	1	0	0	0	2	-2	0	0	1	1	2	0	0	0	1	-1	0	1
Crustaceans	0	0	0	0	1	0	0	0	0	1	0	0	0	0	0	0	0	1	0	1	1
Larvae	1	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	-1	1	1	0
Insect	0	1	1	0	1	0	1	-1	0.5	1	-1	1	1	-0.5	0	0	0	0.5	0	1	0
molluscs	1	0	0	0	1	0	0	0	0	0.5	0.5	0	0	0	0	0	0	0	0	1	1
Fish pursuit	-2	-2	1	0	0	0	2	-2	1	2	-2	1	0	-2	1	0	0	2	-1	1	0
Fish ambush	0	2	2	0	2	0	2	-2	1	2	-2	2	2	-1	0	-1	1	1	0	1	0

To compare the overall capacity to feed on different aquatic food types a PCA was performed on the TPs using R statistical software (3.5.1). For each species, mean TPs were calculated and clustered (using pvclust package in R statistical software (3.5.1), 10 000 bootstrap replicates and the ward.D2 option) to compare feeding capacity between species and by food type, in order to observe which species were most likely to differ.

Results

The minimum and maximum lengths of *C. gilchristi, G. callidus* and *P. knysnaensis* were 17.04 and 84.25 mm, 13.59 and 51.26 mm and 23.45 and 47.93 mm, respectively. The individuals sampled were representative of all size classes for each species. In all three study species, barbels and pharyngeal molariform teeth were absent and therefore not recorded.

Inter species comparisons

The first two dimensions of the PCA ordination of the functional feeding traits of the three species represented only 30.6% of the total variation in the measured functional feeding traits (Figure 4.2). *Glossogobius callidus* is situated towards the bottom left of the ordination, and is characterised by small gape size, velocity suction, hyoid length and relative gape area. *Caffrogobius gilchristi* is situated towards the top left of the ordination and is characterised by high caudal peduncle depth, body depth and lower jaw length. *Psammogobius knysnaensis,* which is situated towards the bottom right of the ordination, is characterised by high gut length. The three species overlap in the centre of the ordination, and those individuals are characterised by average morphology. Individuals with average morphology have high velocity suction and hyoid lengths. *Glossogobius callidus* had the highest degree of overlap with the other two species.

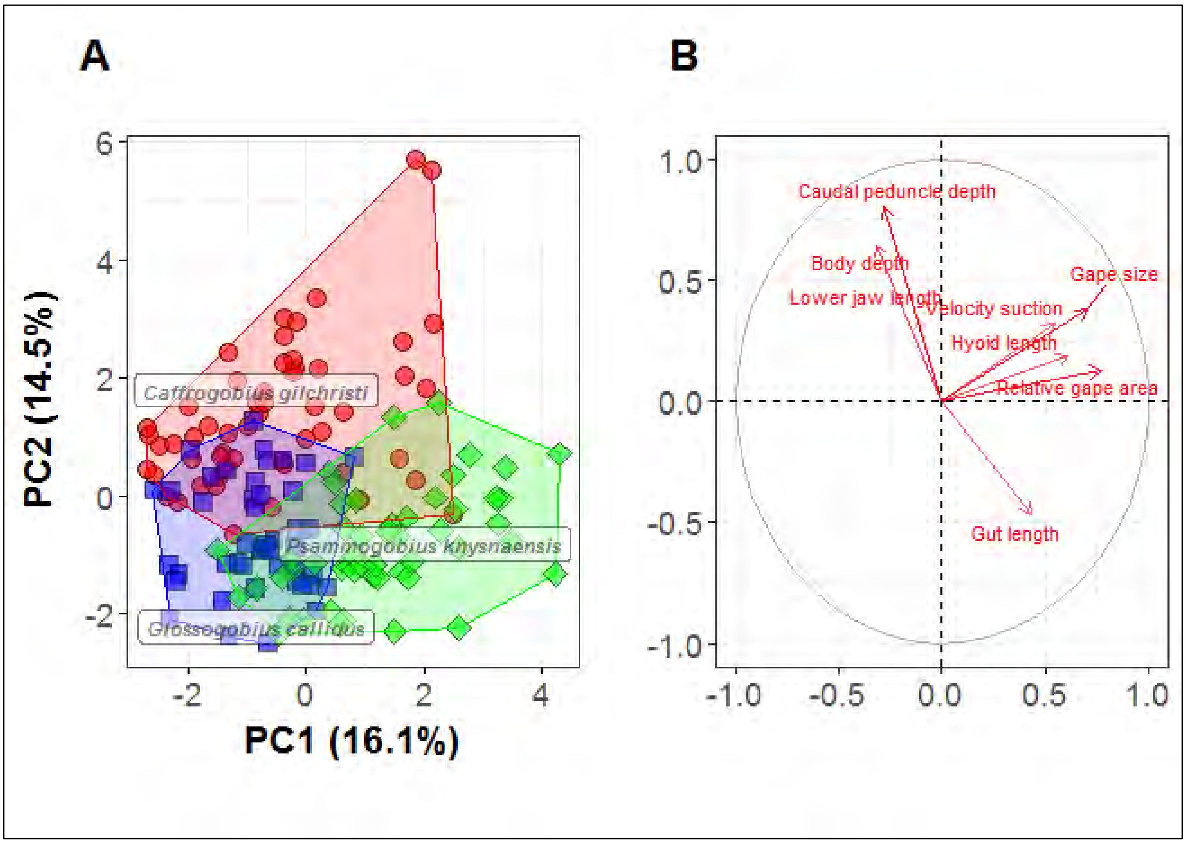


Figure 4.2: Principal component analysis of the 32 measured functional feeding traits of *Psammogobius knysnaensis, Caffrogobius gilchristi* and *Glossogobius callidus* collected from the Sunday Estuary, Eastern Cape, South Africa. A = each marker represents individual fishes and the different colours represent different species. B = direction and size of the loadings of the feeding traits on the ordination

The first two dimensions of the PCA ordination of the TPs represents 72.9% of the total variation (Figure 4.3). Within this ordination, there is a higher degree of overlap between species, particularly between *G. callidus* and *C. gilchristi*. Although *P. knysnaensis* and *C. gilchristi* mainly cluster separately from each other, the trophic niche of *G. callidus* completely overlaps with the other two species. *Caffrogobius gilchristi* potentially feeds on all tested prey (phytoplankton, plants, seeds, detritus, zooplankton, crustaceans, larvae and capturing fish through ambush) except for sessile algae. Individuals of *C. gilchristi* which do not overlap with *G. callidus* are those more capable of consuming insects and molluscs and pursuing fish. However, this does not mean that they cannot consume the other prey, but that they are less likely to consume them compared to the other individuals. *Psammogobius knysnaesis* potentially feeds on sessile algae (although the association is not strong). The position of *Glossogobius callidus* towards the centre of the ordination indicates that it has the most general diet of the three species (Figures 4.3 and 4.4). Trophic profiles showed that *C. gilchristi* are generalist feeders while *P. knysnaensis* and *G. callidus* are specialist feeders (Figure 4.4). The cluster dendrogram shows that *G. callidus* and *P. Knysnaensis* are more likely to feed on similar prey than *C. gilchristi* (Figure 4.5).

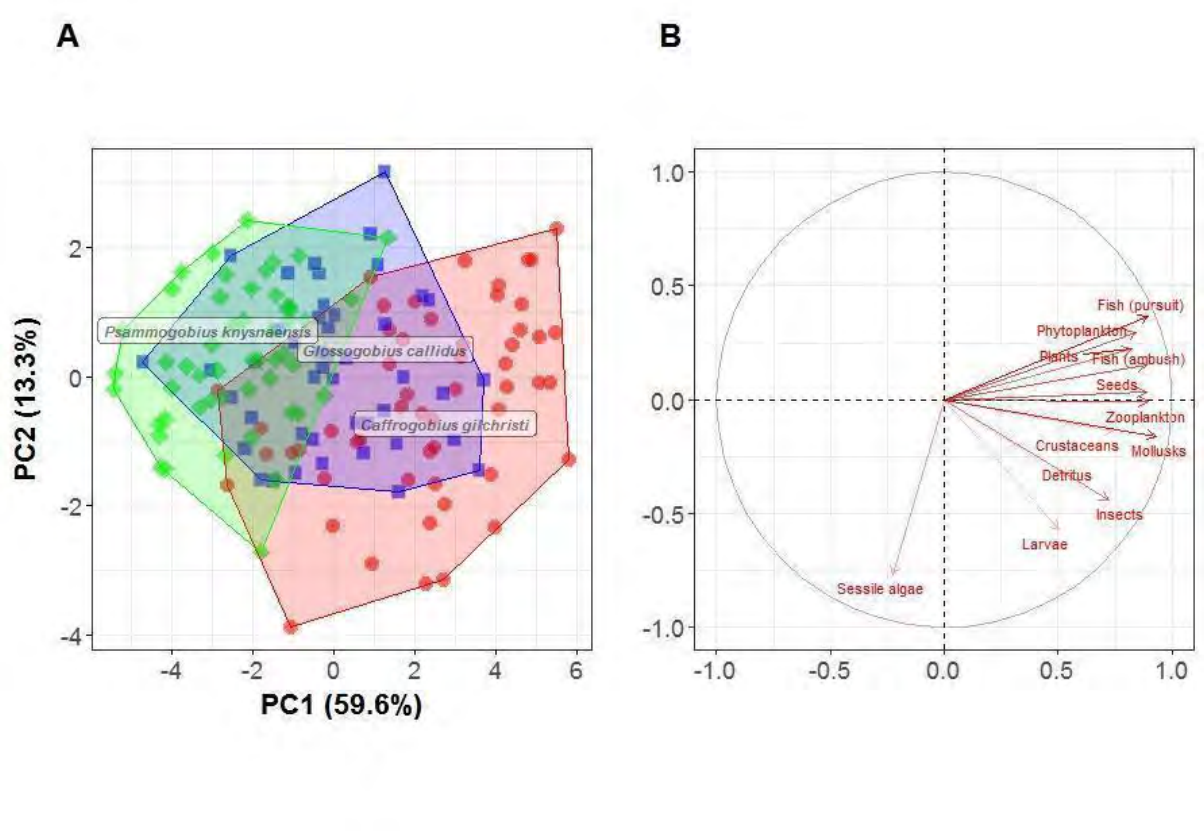


Figure 4.3: Principal component analysis of the trophic profiles of *Psammogobius knysnaensis, Caffrogobius gilchristi* and *Glossogobius callidus* collected from the Sunday Estuary, South Africa. In the left panel (A) different colours represent different species and each marker represents an individual. The right panel (B) represents the sizes of the loading and directions of food specialist profiles

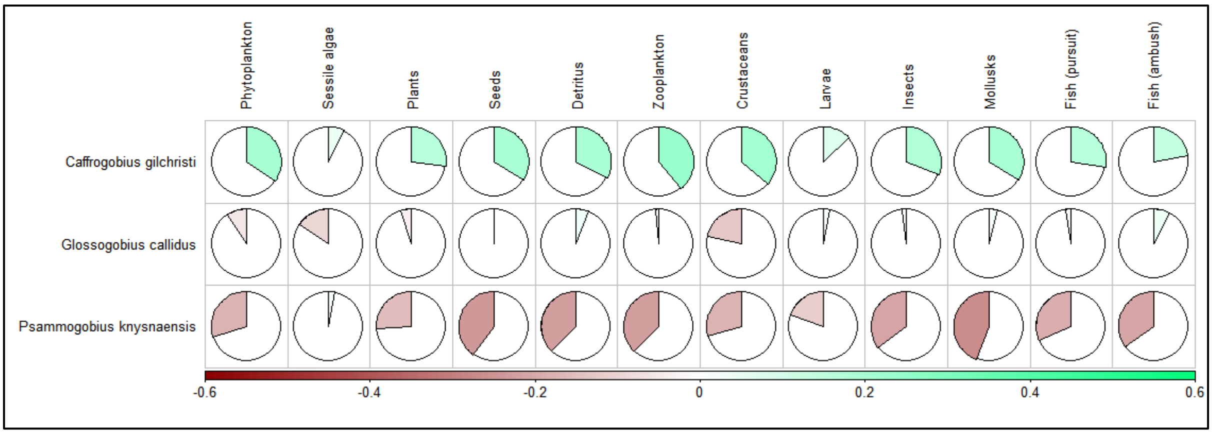


Figure 4.4: Mean trophic profiles per fish species showing their capability to consume aquatic food types. Pies = mean correlations of the species morphology with proposed profiles for each food resource specialist. Green = positive correlation; red = negative correlation

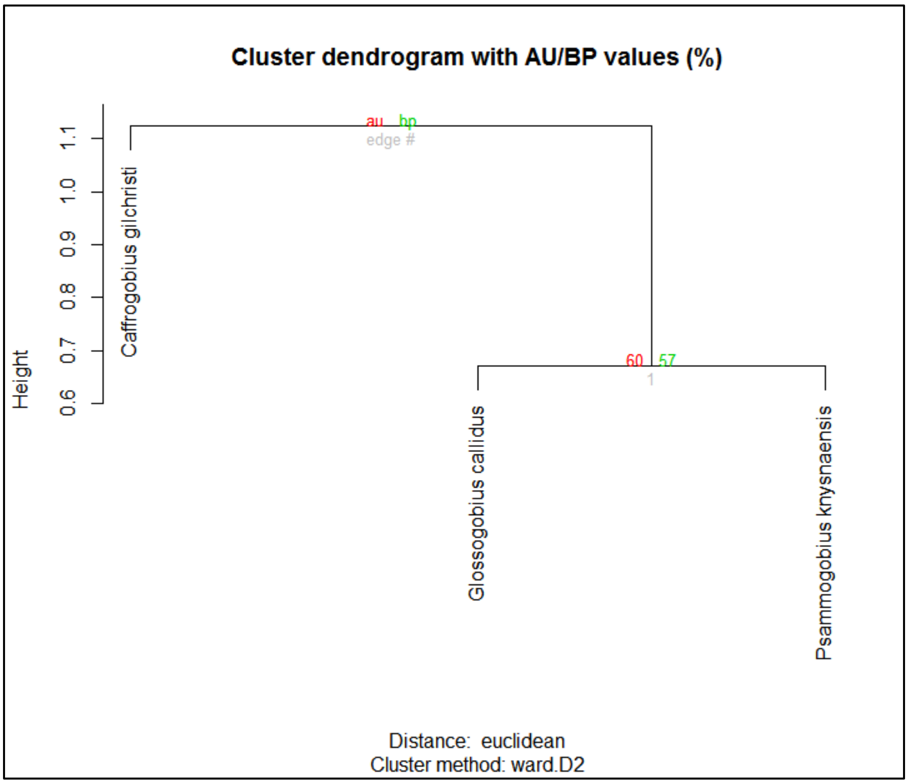


Figure 4.5: Dendrogram showing trophic profiles clustering of *Psammogobius knysnaensis, Caffrogobius gilchristi* and *Glossogobius callidus* collected from the Sundays Estuary, South Africa

Discussion

In terms of both morphological traits and trophic profiles, *C. gilchristi* and *P. kysnaensis* are fairly distinct from each other. In contrast, the morphological traits and trophic profiles of *G. callidus* overlapped with the other two species. This suggests that the diet of *C. gilchristi* and *P. kysnaensis* would also be different, thus reducing competition for food and promoting co-existence. Where closely related species occupy the same habitat, they tend to have different feeding morphological traits or feed in different levels of the water column (Stoner and Livingston, 1984; Kabasakal, 2001; Adams and Huntingford, 2002). This results in the evolution of different feeding specialisations, allowing them to coexist under such conditions (Adams and Huntingford, 2002). For example, in a study by Stoner and Livingstone (1984), two closely related sparids pinfish, *Lagodon rhomboides* and spottail seabream *Diplodus holbrooki*, from Apalachee Bay, Florida had limited dietary overlap and were morphologically different. The former specialised in prey that required mastication such as microepiphytes, sponges and hydroids, while the later specialised in non-masticated amphipods, shrimps and isopods. *Diplodus holbrooki* had smaller mouths and sharper incisors than the other species, and were unable to consume large prey (Stoner and Livingstone, 1984).

In contrast to *P. knysnaensis* and *C. gilchristi*, the feeding morphological traits and trophic niche of *G. callidus* overlapped highly with both species, indicating high dietary overlap. This indicates the potential to compete with both species for food in the wild. In correlation, in a study by Clifton and Motta (1998) in Tennessee Reef (Florida), two Labridae species with low crushing force (*Halichoeres maculipinna* and *Thalassoma bifisciatum*) had similar diets, and those with hard crushing forces (*H. garnoti* and *H. bivittatus*) also had similar diets. Although the diet of *Glossogobius callidus* may be similar to the other two species, studies in estuaries suggest that this type of co-existence occurs in the upper reaches of estuaries, whereas specialisation appears to be more abundant in the lower reaches (Richardson et al., 2006; Bailey and James, 2013; Tweddle and Froneman, 2015; Nodo et al., 2017). Prey availability in the feeding environments could result in these differences.

Because the morphological traits of an organism are linked to its diet, morphological changes between species can explain interspecific and ontogenetic differences in their diets (Wainwright, 1988; Wainwright and Richard, 1995; Mittelbach et al., 1999; Sibbing and Nagelkerke, 2001; Nagelkerke, 2018). *Psammogobius knysnaensis* is characterised by a long gut, gape area, hyoid length, relative gape area, and high velocity suction, suggesting that large prey will dominate its diet. Species with large mouth gape consume larger prey than species with small mouth gape (Wainwright and Richard, 1995). The long guts observed in *P. knysnaensis* also suggest that this species has a longer digestion time, and therefore feeds less often than the other two species. In a study by Hofer and Schiemer (1981), species with longer guts took more time to digest their prey and had high absorption, as this allows more time for nutrient absorption in the body. Long guts are mostly associated with herbivorous fish (Hofer and Schiemer, 1981) and this suggests that there is a possibility that this species is capable of consuming plants in the wild.

Caffrogobius gilchristi differs from the other species by a large body depth, lower jaw length and caudal peduncle depth. Body form affects lifting, weight, drag, and friction, and all of these determine prey capture speed (Webb et al., 1996; Webb, 2002). In a study by Fisher and Hogan (2007), fishes with narrow caudal peduncle depths and large body depths were found to have higher swimming speed than fishes with large caudal peduncle depth and small body depth. This implies that under competitive situations, *C. gilchristi* may outcompete *P. kysnaensis* and *G. callidus* for food.

Morphological overlapping individuals of the three species were characterised by high lower jaw length, hyoid length and velocity suction. These morphological traits play an important role in prey intake and mouth closing (Richard and Wainwright, 1995). During mouth opening for prey intake, jaws need to be in their maximum displacement in order to reach maximum gape, and for mouth closing, adduction of the lower jaw and dorsal movement of the hyoid must occur (Richard and Wainwright, 1995). The longer the jaw, the quicker the fish could potentially open and close its mouth during prey capture (Richard and Wainwright, 1995). All the above features affect the capacity of these organisms to feed on different food resources.

Conclusion

As hyposythesised, this study shows that there is little similarity in the trophic morphology of *P. knysnaensis* and *C. gilchristi,* while that of *G. callidus* is similar to both species. This suggests different feeding capacity and resource partitioning for the first two species, while *G. callidus* is predicted to compete with both species for food in the wild. These findings will be further investigated in the dietary analysis in the next chapter.

CHAPTER FIVE

Feeding ecology of *Psammogobius knysnaensis, Caffrogobius gilchristi* and *Glossogobius callidus* from the Sundays Estuary

Introduction

Patterns of resource use are a useful tool for measuring resource partitioning between coexisting species. Closely related species co-occurring in the same habitat are likely to compete for resources such as space, shelter and food (Borza et al., 2009; Kramer et al., 2009). In ecological communities, co-occurring fish species reduce their potential for interspecific competition by partitioning their resources (Hyndes, 1997), with different species tending to specialise in different prey, or feeding on the same prey but in different habitats, when prey is scarce (Saeki et al., 2005; Pimentel and Joyeux, 2010; Stevove and Kovac, 2013; Su et al., 2016). However, when resources are abundant, reduced competition may result in increased dietary overlap (Horn, 1983).

Prey availability fluctuates with season or habitat, predator size, and the abundance of conspecific and interspecific competitors (Ogle et al., 1995; Greenstreet et al., 1998; Beukers-Stewart and Jones, 2004). As fish consume prey in relation to availability, fish diet can be correlated with change in prey composition between habitats, together with their abundance in those habitats (i.e., low prey abundance results in low representation in the diet and, conversely, high prey abundance will result in higher representation) (Menge, 1972; Buckel et al., 1999; Trenkel, et al. 2003). As a result, the extent of resource partitioning can vary seasonally and even diurnally (Scrimgeour and Winterbourn, 1987; White and Potter, 2004).

It is also common for fish diets to change with size, and usually when they are small, they consume small sized prey, and switch to larger prey as they grow (Skora and Rzeznik, 2001; Gkenas el at., 2012; Guo et al., 2014). This behaviour has been observed in the boshi goby *Rhinogobius cliffordpopei* (Nichols, 1925) and oriental river-goby *Rhinogobius giurinus* (Nichols, 1925), where both species specialised in consuming macrozooplankton as juveniles and insects and Tubificidae as sub-adults and adults (Guo et al., 2014). Similarly, round goby *Neogobius melanostomus* changes its diet from soft prey (crustaceans, Gammaridae and Polychaeta) as a juvenile to hard shell prey as an adult (Mollusca and Bivalvia) (Skora and

Rzeznik, 2001). This dietary change with fish size was also observed in the river goby *Glossogobius callidus* (Smith, 1937) in the Sundays River irrigation ponds (Mofu et al., 2019). Although both juvenile and adult *G. callidus* are generalist feeders feeding on dipterans, Mofu et al. (2019) noted that they partitioned their food resource by size, with juveniles and adults specialising on different dipterans prey. Similarly, in estuary studies, the consumption of copepods by both *P. knysnaensis* and *G. callidus* is known to decrease with an increase in fish size (Bennett, 1989; Vumazonke, 2008; Wasserman, 2012). During the adult stages of *G. callidus*, amphipods constitute a high proportion of their diet (Vumazonke et al., 2008). *Caffrogobius gilchristi* was observed to shift their diet from feeding on cyclopoids as juveniles, to favouring amphipods and insect larvae during late juvenile and adult stages (Bennett, 1989). The niche breadth parameter is used to understand this dietary specialization (Sá-Oliveira et al., 2014).

Fish size is another factor that determines dietary overlap between co-occurring species. For example, Bluegill *Lepomis macrochirus* (Rafinesque, 1810) and Pumpkinseed *Lepomis gibbosus* (Linnaeus, 1758) had more dietary similarity during their early developmental stages than at adult stages in Lawrence Lake, Michigan (Mittelbach, 1984). These closely related species are equal competitors during early developmental stages as they both feed on vegetated habitats during this stage. During adult stages, foraging efficiency and functional morphological changes occur (the development of fine needle like pharyngeal teeth for *L. macrochirus* and strong molar form teeth for *L. gibbosus*), enabling the former to feed more on zooplankton, while the latter feeds more on snails (Mittelbach, 1984).

The morphological comparison between *P. knysnaensis* and *C. gilchristi* (Chapter 4) showed that the two species had different overall morphology with little overlap. Based on these results, *P. knysnaensis* was predicted to feed on large and hard prey. In corroboration, Whitfield (1988) and Bennett and Branch (1990) found that *P. knysnaensis* in the Wilderness and Bot Estuaries is a carnivorous benthic feeder feeding on amphipods, insects and polychaeta. *Caffrogobius gilchristi* is characterised by large body depth, and lower jaw length and caudal peduncle depth. These characteristics promote high swimming speed during prey capture, suggesting that it outcompetes *P. knysnaensis* in competitive situations, as copepods are common in the diet of both species (Webb et al., 1996). In the Bot Estuary, *C. gilchristi* has been observed to shift its diet from ostracods and copepods to amphipods and decapods (Bennett, 1989). The overall feeding morphology of *G. callidus* overlapped with that of both *P. knysnaensis* and *C.*

gilchristi. This suggests that the diet of *G. callidus* could overlap with that of both *P. knysnaensis* and *C. gilchristi*. Seasonal analysis of *G. callidus* in freshwater pond environments has shown that it is a benthic generalist feeder, with Tanypodinae, Orthocladiinae and Chironominae in common (Mofu et al., 2019). In estuarine environments *G. callidus* specializes in consuming amphipods and copepods in all seasons (Vumazonke et al., 2008; Wasserman et al., 2012).

In this chapter, the diet and ontogenetic dietary shifts of the three species in the Sundays Estuary were investigated. The central hypothesis was that the feeding ecology of these species would be consistent with predictions from the functional morphology study, and with previous studies on the diet of these species. Based on the morphology results, *P. knysnaensis* was expected to feed on large and hard prey. *Psammogobius knysnaensis* and *C. gilchristi* were predicted to have different diets, as their overall functional morphology is different. *Glossogobius callidus*' diet was predicted to overlap with that of *P. knysnaensis* and *C. gilchristi*, as their overall functional morphology also overlapped.

Materials and Methods

Field sampling

Sampling was conducted twice, in spring (September/October 2018) and in winter (August 2019). This sampling period is consistent with late winter/early spring in the Eastern Cape. Sampling was done in the nine sampling sites and then divided by the lower and the upper section. A total of 99 individuals of *P. knysnaensis,* 104 *C. gilchristi* and 104 *G. callidus* were collected during the 2018 survey. In the 2019 survey, a total of 173 individuals of *P. knysnaensis,* 81 *C. gilchristi* and 49 *G. callidus* were collected. Fish samples were collected by seine netting (30 m x 2 m with a 5 mm bar mesh) in the littoral zone and beam trawling in the channel of the estuary. During sampling, the seine net was deployed in a semicircle from the bank using a boat and then hauled to the bank by three or four people. The beam trawl was 1.5 m wide and conical, tapering to 0.5 m in the cod end, with 6.5 mm knotless nylon mesh at the end of the net (Nodo et al., 2018). During sampling, the beam trawl net was towed 20 m behind the boat at a speed of ~2 knots for three minutes. One trawl was done at each of the nine sites described in Chapter 2, covering a distance of approximately 200 m. After each trawl, fish were identified and sorted. Only the three study species were kept, all other species were released. After capture, fish were immediately placed in a bucket with an overdose of 40 mg l-1 clove oil for humane euthanasia (Rhodes university ethical clearance number 2019-0543-750) (Neiffer and Stamper, 2009). Once dead, the fish were placed in a cooler box with ice until they reached the laboratory (Ocean Science NMU) on the same day.

Laboratory analysis

In the laboratory, each fish was measured for total length (TL) to the nearest mm and the stomach of the fish then removed via dissection. Fish stomachs were stored separately in 10% formalin solution for a period of one week for fixation before being transferred to 70% ethanol. Each stomach was dissected and the contents were emptied into a customised 5 mm deep dissecting tray with grid markings at increments of 1 mm x 1 mm to allow for volume estimation (Wasserman et al., 2011). The stomach contents were identified to various taxonomic levels (preferably family level), depending on the group and/ or level of digestion. Keys used were body shapes, body segment, mouth parts and appendages depending on the prey group. This was done under a dissecting microscope using marine and estuarine invertebrate identification guides, following the recommendations of Hyslop (1980). Prey counts were based on heads as the other body parts were digested. The volume of each prey taxa was determined using an indirect volumetric method, where prey was squashed to a uniform depth within the dissection tray and the volume calculated as the grid-area covered.

Analysis

To determine ontogenetic diet shifts and dietary comparisons between species, fish were divided into length classes of < 20 mm, 20–29 mm, 30–39 mm, 40–49 mm, 50–59 mm, 60–69 mm, and > 70 mm. Prey abundance (%N), frequency of occurrence (%F) and percentage volume (%V) was calculated for each dietary prey in each fish species. %N is defined as the number of individuals as the proportion of all prey items, %F represents the number of stomachs containing a particular prey as a percentage of all stomachs and %V is the relative contribution of each prey type in relation to the total volume of all stomach contents (using the methods detailed in Pinkas et al., 1971). In addition, the relative importance of each invertebrate taxon was assessed using the index of relative importance (IRI).

$$IRI = (\%N + \%V) \times \%F$$

For comparative purposes, the IRI value for each group was expressed as a proportion of the sum of IRI values calculated for all prey items (%IRI). Index of relative importance was calculated for the identifiable prey taxa, but not unidentified material such as detritus and vegetation. Length sizes where the contribution of ostracods and cyclopoids was less than 50% (IRI) were defined as small individuals, and large individuals were represented by greater than 50% (IRI) contribution.

The %IRI data was used to construct a dietary niche breadth for each size class per species using the equation:

$$B = \frac{1}{\Sigma Pi^2}$$

Where B = The niche breadth, Pi is the relative frequency for prey item i in the diet of predator P (Levins, 1968). The results were then standardized to the scale of 0–1 using the equation:

$$B_{\mathrm{a}} = (B - 1)(n - 1)$$

Where B_a = standardised niche breadth, and n = the summation number of all the prey items. Niche overlap between fish size classes was calculated using the formula:

$$Ojk = \frac{\Sigma PijPik}{\sqrt{\Sigma P2ijP2ik}}$$

where Ojk is the measure of overlap between size class j and size class k for species, Pij is the proportion that resource i is of the total resources used by size class j, and Pik is the proportion that resource i is of the total resources used by size class k (Pianka, 1986).

To conduct further analysis on the dietary similarities of co-existing species length classes, %IRI values were square root transformed. Cluster analysis was plotted to visualise the similarity between gut content analysis structure of the three species using a Bray-Curtis similarity matrix in PRIMER v6. SIMPER analysis was then used to determine the contribution of each prey taxa to the diet.

A modified Costello graphical method (Costello, 1990; Amundsen et al., 1996) was used to monitor the feeding behaviour of the study species. In this method, prey-specific abundance (percentage of prey taxa) was plotted against frequency of occurrence. This was determined using the equation

$$p_i = 100(\Sigma \quad S_i)(\Sigma \quad S_{ti})^{-1}$$

where p_i is the prey specific abundance of prey i, S_i the stomach content composed of prey i, and S_{ti} the total stomach content in only those fish with prey i in their stomachs. The position of the prey in Figure 5.1 was used to explain prey importance, feeding strategy and niche width contribution.

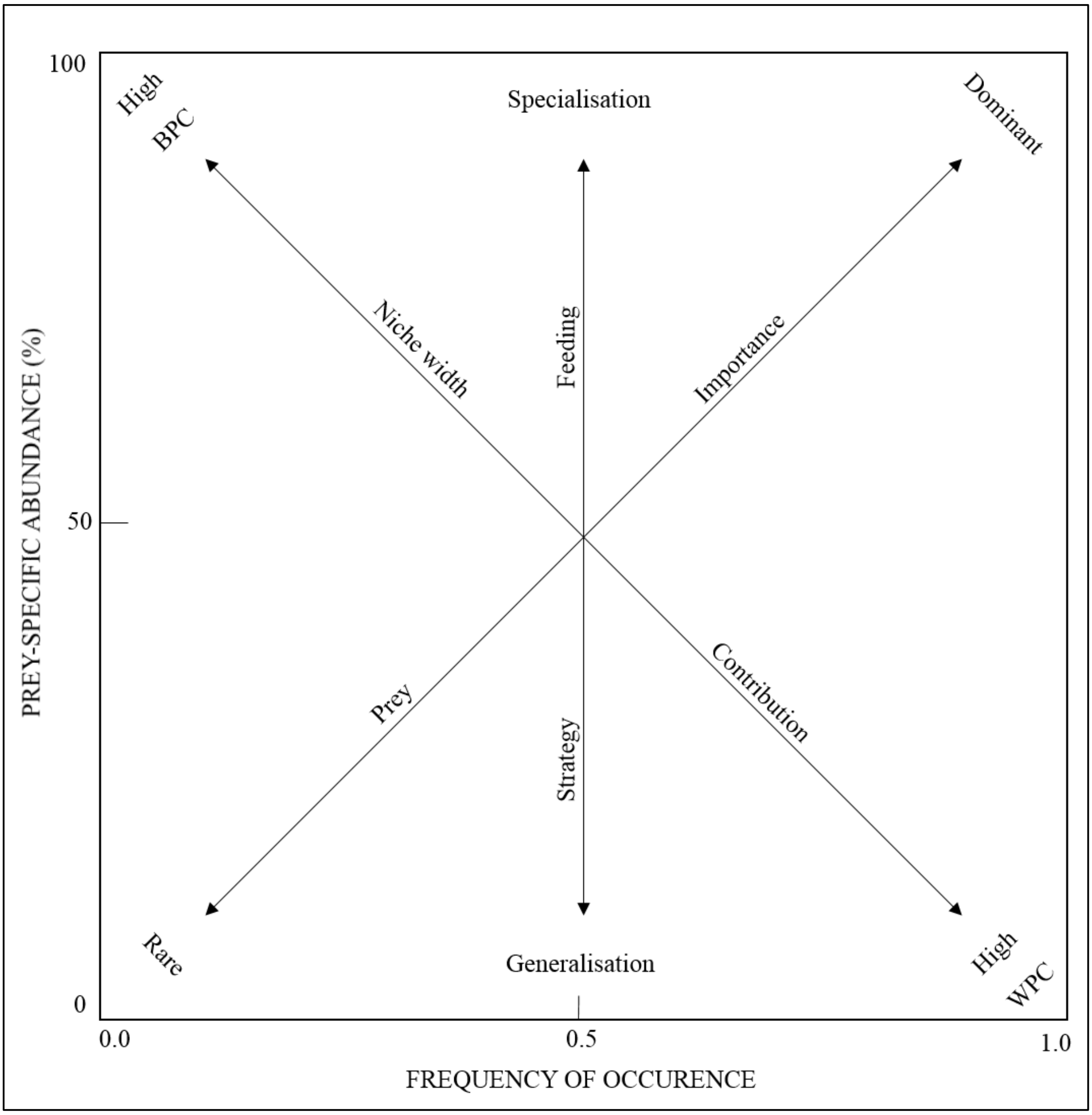


Figure 5.1: Diagram for interpretation of prey importance, niche width contribution and feeding strategy as described by Amundsen et al. (1996). Niche width contribution = high BPC/high WPC. High between phenotype components (BPC) represents a situation where different individuals specialise in different types of resource. High within-phenotype component (WPC) occurs when most of the individuals utilise many resource types. Prey importance = dominant/rare, feeding strategy = specialisation/generalised

Results

A total of 99, 104 and 104 individuals of *P. knysnaensis, C. gilchristi* and *G. callidus* were collected during the 2018 survey. In the 2019 survey, a total of 173, 81 and 49 individuals of *P. knysnaensis, C. gilchristi* and *G. callidus* were collected.

Psammogobius knysnaensis

Diet and ontogenetic shifts

In total, 276 stomachs were examined for *P. knysnaensis*. Of the stomachs analysed only two were empty. A total of 13 prey taxa represented the diet of this species, with ostracods and cyclopoids dominating the diet of this species both in %IRI and %N (Figure 5.2 and Table 5.1).

To investigate the effect of size on fish diet, fish were grouped into 10 mm size classes. Prey consumption significantly changed ($\chi^2 = 377.8$, df = 48, $p < 0.05$) with fish size (Figure 5.2 and Table 5.1). Although ostracod and cyclopoid consumption were high in all size classes, fish larger than 39 mm TL had a slightly different diet, with ostracod and cyclopoid contribution less than 50% (%IRI and %N). An ontogenetic shift in diet occurred from 30–39 mm TL, with ostracods and cyclopods together comprising less than 50% of the diet (<50% IRI) (Figure 5.2). In individuals of 40–49 mm TL, bivalves contributed 47.1% IRI. This prey item was only recorded in the stomachs of individuals larger than 30 mm. Amphipod consumption increased with fish size, with amphipods recorded in the largest numbers in the largest size class (60–69 mm TL) (%IRI = 33.78%). Chironomids (%IRI = 4.17%) were also most prevalent in the diet of the largest size class (Figure 5.2 and Table 5.1).

Table 5.1: Gut contents of *Psammogobius knysnaensis, Caffrogobius gilchristi* and *Glossogobius callidus* sampled in September/October 2018 and August 2019 from the Sundays River Estuary, Eastern Cape, South Africa. All prey items were identified to lowest possible taxon (%N is the number of individuals as the proportion of all prey items, %F is the percentage of all stomachs containing prey, %V is the volume of each prey item consumed, as a percentage of the total volume of stomach contents; %IRI index of relative importance, as a proportion of the total IRI of all species sampled). Bold values indicate high %IRI contribution. n = fish number, n 2 = prey number and v = volume

			Psammogobius knysnaensis							***Caffrogobius gilchristi***							***Glossogobius callidus***					
Size	**n**	**Prey Taxa**	**v**	**n 2**	**%N**	**%F**	**%V**	**% IRI**	**n**	**v**	**n 2**	**%N**	**%F**	**%V**	**% IRI**	**n**	**v**	**n 2**	**%N**	**%F**	**%V**	**% IRI**
<20	5	Ostracoda	17.0	9	32.1	40.0	28.1	**28.9**	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		Cyclopoida		7	25.0	60.0	53.1	**56.3**			-	-	-	-	-			-	-	-	-	-
		Chironomidae		2	7.1	20.0	3.1	2.5			-	-	-	-	-			-	-	-	-	-
		Amphipoda		2	7.1	20.0	3.1	2.5			-	-	-	-	-			-	-	-	-	-
		Cyclopoid eggs		2	7.1	20.0	3.1	2.5			-	-	-	-	-			-	-	-	-	-
		Mysidae		2	7.1	20.0	3.1	2.5			-	-	-	-	-			-	-	-	-	-
		Baetidae		2	7.1	20.0	3.1	2.5			-	-	-	-	-			-	-	-	-	-
		Brachyura		2	7.1	20.0	3.1	2.5			-	-	-	-	-			-	-	-	-	-
20–29	50	Ostracoda	100.5	211	58.0	42.0	41.4	**68.9**	16	38	12	8.9	18.8	216.7	**4.4**	26	54	10	7.5	3.9	0.9	**0.4**
		Cyclopoida		119	32.7	26.0	28.2	**26.1**			113	83.7	75.0	1040.0	**87.8**			97	72.9	50.0	48.9	**72.8**
		Chironomidae		4	1.1	6.0	5.7	0.7			1	0.7	6.3	33.3	0.2			5	3.8	15.4	9.2	2.4
		Amphipoda		8	2.2	8.0	5.0	1.0			3	2.2	12.5	53.3	0.7			15	11.3	46.2	30.7	23.1
		Mysidae		2	0.6	2.0	2.8	0.1			0	0.0	0.0	0.0	0.0			0	0.0	0.0	0.0	0.0
		Baetidae		0	0.0	0.0	0.0	0.0			0	0.0	0.0	0.0	0.0			1	0.8	3.9	1.0	0.1
		Brachyura		1	0.3	2.0	1.2	0.1			6	4.4	25.0	256.7	**6.8**			0	0.0	0.0	0.0	0.0
		Bivalve		4	1.1	6.0	5.5	0.7			0	0.0	0.0	0.0	0.0			0	0.0	0.0	0.0	0.0
		Gastropoda		1	0.3	2.0	1.4	0.1			0	0.0	0.0	0.0	0.0			5	3.8	7.7	9.4	1.2

		Cumacea: bodotriidae		14	3.9	12.0	8.8	2.5			0	0.0	0.0	0.0	0.0			0	0.0	0.0	0.0	0.0
30–39	112	Ostracoda	780.3	518	34.7	24.1	16.9	**22.7**	49	175	43	8.5	14.3	4.8	**1.4**	35	122.75	0	0.0	0.0	0.0	0.0
		Cyclopoda		656	44.0	42.9	42.5	**67.7**			429	84.8	83.7	77.9	**97.3**			138	62.3	51.4	32.3	**53.7**
		Chironomidae		7	0.5	4.5	0.7	0.1			0	0.0	0.0	0.0	0.0			37.6	17.0	40.0	22.7	**17.5**
		Amphipoda		58	3.9	11.6	7.6	2.4			14	2.8	6.1	5.3	0.4			27	12.2	48.6	28.0	**21.5**
		Cyclopoid eggs		87	5.8	1.8	0.2	0.2			0	0.0	0.0	0.0	0.0			0	0.0	0.0	0.0	0.0
		Mysidae		8	0.5	5.4	6.2	0.7			1	0.2	2.0	1.5	0.0			0	0.0	0.0	0.0	0.0
		Baetidae		0	0.0	0.0	0.0	0.0			0	0.0	0.0	0.0	0.0			19	8.6	25.7	17.1	**7.3**
		Brachyura		9	0.6	5.4	2.8	0.3			5	1.0	10.2	5.0	0.4			0	0.0	0.0	0.0	0.0
		Fish larvae		1	0.1	0.9	0.7	0.0			0	0.0	0.0	0.0	0.0			0	0.0	0.0	0.0	0.0
		Bivalve		110	7.4	13.4	12.9	5.0			0	0.0	0.0	0.0	0.0			0	0.0	0.0	0.0	0.0
		Gastropoda		8	0.5	1.8	1.2	0.1			14	2.8	8.2	5.5	0.5			0	0.0	0.0	0.0	0.0
		Polychaeta		9	0.6	2.7	2.3	0.1			0	0.0	0.0	0.0	0.0			0	0.0	0.0	0.0	0.0
		Cumacea: bodotriidae		20	1.3	6.3	4.8	0.7			0	0.0	0.0	0.0	0.0			0	0.0	0.0	0.0	0.0
		Calanoid		0	0.0	0.0	1.1	0.0			0	0.0	0.0	0.0	0.0			0	0.0	0.0	0.0	0.0
40–49	81	Ostracoda	1283.5	623	38.0	21.0	10.2	**16.8**	71	730.25	48	6.1	15.5	2.6	1.8	31	251	1	0.3	3.2	0.1	0.0
		Cyclopoida		476	29.0	32.1	20.4	**26.3**			486	61.6	57.8	48.3	**82.8**			210	52.6	54.8	27.4	**39.3**
		Chironomidae		9	0.6	6.2	0.8	0.1			16	2.0	7.0	2.4	0.4			62	15.5	67.7	22.3	**23.0**
		Amphipoda		67	4.1	16.1	9.0	3.5			64	8.1	14.1	7.8	2.9			98	24.6	61.3	35.4	**32.9**
		Mysidae		31	1.9	17.3	11.8	3.9			39	4.9	23.9	23.8	**9.0**			0	0.0	0.0	0.0	0.0
		Baetidae		1	0.1	1.2	0.5	0.0			0	0.0	0.0	0.0	0.0			24	6.0	25.8	14.4	**4.7**
		Brachyura		12	0.7	8.6	4.0	0.7			11	1.4	9.9	5.2	0.8			1	0.3	3.2	0.2	0.0
		Fish eggs		0	0.0	0.0	0.0	0.0			58	7.4	2.8	1.9	0.3			0	0.0	0.0	0.0	0.0

		Fish larvae		1	0.1	1.2	0.5	0.0			0	0.0	0.0	0.0	0.0			0	0.0	0.0	0.0	0.0
		Bivalve		376	22.9	46.9	37.6	**47.1**			0	0.0	0.0	0.0	0.0			0	0.0	0.0	0.0	0.0
		Gastropoda		28	1.7	18.5	2.6	1.3			58	7.4	11.3	4.5	1.7			0	0.0	0.0	0.0	0.0
		Polychaeta		8	0.5	1.2	0.7	0.0			1	0.1	1.4	1.0	0.0			0	0.0	0.0	0.0	0.0
		Cumacea: bodotriidae		8	0.5	6.2	2.0	0.3			4	0.5	1.4	0.8	0.0			0	0.0	0.0	0.0	0.0
		Glyceridae		0	0.0	0.0	0.0	0.0			4	0.5	5.6	1.8	0.2			0	0.0	0.0	0.0	0.0
		Calanoid		0	0.0	0.0	0.0	0.0			0	0.0	0.0	0.0	0.0			3	0.8	6.5	0.2	0.1
50–59	28	Ostracoda	478.0	103	29.9	25.0	4.5	**12.8**	18	238.75	22	11.2	11.1	1.5	**3.1**	28	254.75	2	0.7	3.6	0.1	0.0
		Cyclopoida		121	35.1	50.0	19.1	**40.5**			69	35.2	27.8	16.2	**31.4**			140	45.5	39.3	22.1	**26.4**
		Chironomidae		12	3.5	28.6	6.2	**4.1**			3	1.5	11.1	2.2	0.9			54	17.5	71.4	28.8	**32.9**
		Amphipoda		64	18.6	42.9	34.2	**33.8**			47	24.0	16.7	16.5	**14.8**			97	31.5	57.1	36.5	**38.7**
		Mysidae		4	1.2	10.7	7.8	1.4			7	3.6	33.3	32.1	**26.2**			1	0.3	3.6	3.2	0.1
		Baetidae		3	0.9	10.7	5.2	1.0			0	0.0	0.0	0.0	0.0			8	2.6	17.9	6.3	1.6
		Brachyura		5	1.5	10.7	11.0	2.0			8	4.1	27.8	15.1	**11.7**			0	0.0	0.0	0.0	0.0
		Fish larvae		0	0.0	0.0	0.0	0.0			0	0.0	0.0	0.0	0.0			1	0.3	3.6	2.3	0.1
		Bivalve		25	7.3	14.3	10.7	3.8			1	0.5	5.6	4.2	0.6			0	0.0	0.0	0.0	0.0
		Gastropoda		8	2.3	10.7	1.3	0.6			36	18.4	16.7	11.9	**11.1**			0	0.0	0.0	0.0	0.0
		Glyceridae		0	0	0	0	0			3	1.5	5.6	0.4	0.2			0	0.0	0.0	0.0	0.0
		Calanoid		0	0	0	0	0			0	0.0	0.0	0.0	0.0			5	1.6	7.1	0.7	0.2
60–69	-	Cyclopoida	-	-	-	-	-	-	-	-	-	-	-	-	-	19	228.25	28	14.7	31.6	7.6	**5.1**
		Chironomidae		-	-	-	-	-			-	-	-	-	-			55	29.0	68.4	23.8	**26.0**
		Amphipoda		-	-	-	-	-			-	-	-	-	-			86	45.3	89.5	55.0	**64.6**
		Mysidae		-	-	-	-	-			-	-	-	-	-			1	0.5	5.3	5.1	0.2

		Baetidae		-	-	-	-	-			-	-	-	-	-			18	9.5	31.6	8.2	**4.0**
		Brachyura		-	-	-	-	-			-	-	-	-	-			2	1.1	5.3	0.5	0.1
>70	-	Cyclopoida	-	-	-	-	-	-			-	-	-	-	-	7	87.75	3	7.9	14.3	0.9	1.6
		Chironomidae		-	-	-	-	-			-	-	-	-	-			13	34.2	42.9	35.5	**37.2**
		Amphipoda		-	-	-	-	-			-	-	-	-	-			12	31.6	57.1	35.4	**47.6**
		Mysidae		-	-	-	-	-			-	-	-	-	-			3	7.9	14.3	10.8	**3.3**
		Baetidae		-	-	-	-	-			-	-	-	-	-			5	13.2	28.6	9.0	**7.9**
		Calanoid		-	-	-	-	-			-	-	-	-	-			2	5.3	14.3	8.3	2.4

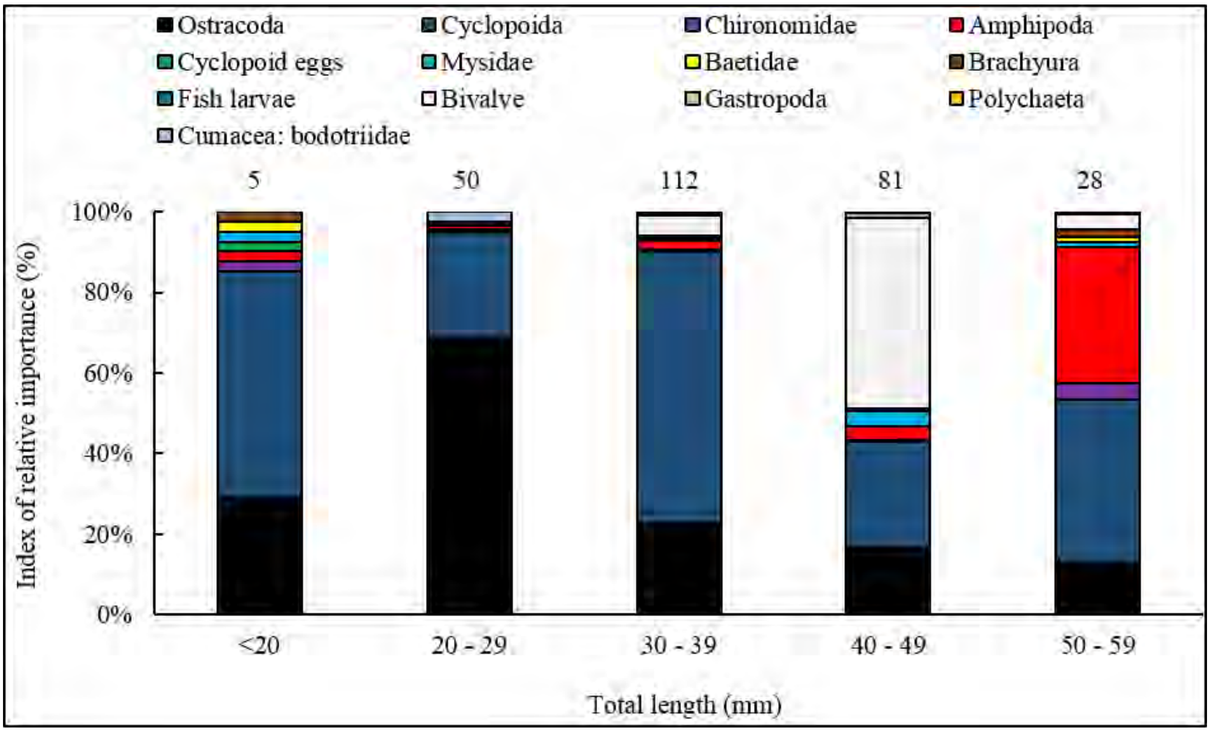


Figure 5.2: Percentage index of relative importance (%IRI) of prey items found in the stomachs of different size classes of *Psammogobius knysnaensis* from combined years in the Sundays Estuary, Eastern Cape, South Africa. Numbers above bars represent sample number values

Niche breadth was highest in the smallest (< 20 mm) and the largest size classes (50–59 mm) (Figure 5.3). The smallest size class consumed eight prey taxa, namely, ostracoda, cyclopoida, chironomidae, amphipoda, cyclopoid eggs, mysidae, baetidae and brachyura. The largest size consumed nine prey taxa, namely, ostracoda, cyclopoida, chironomidae, amphipoda, mysidae, baetidae, brachyura, bivalve and gastropoda. Bivalve, gastropoda and cumacea: bodotriidae were only consumed by individuals greater than 20 mm in length.

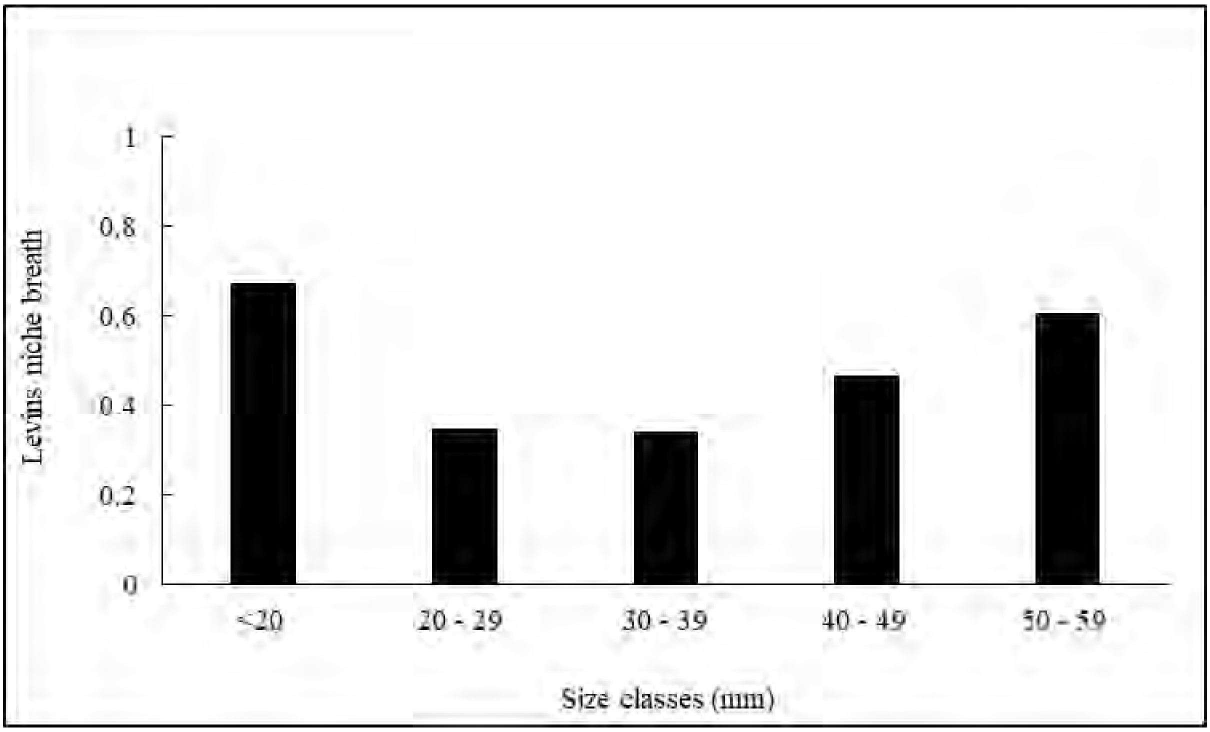


Figure 5.3: Dietary Levins niche breath of *Psammogobius knysnaensis* size classes collected from the Sundays Estuary, Eastern Cape South Africa

Feeding strategy

Psammogobius knysnaensis individuals collected in both sampling periods were generalist feeders, feeding on pelagic ostracods and cyclopoids (Figure 5.4). As all consumed prey had a prey-specific abundance of below 50%, it cannot be concluded that the species has a specific diet, and so it is referred to as a generalist feeder. This species was an opportunistic feeder on chironomidae, amphipoda, cyclopoid eggs, mysidae, baetidae, brachyuran, fish larvae, bivalves, gastropods, polychaeta and cumacea: bodotriidae.

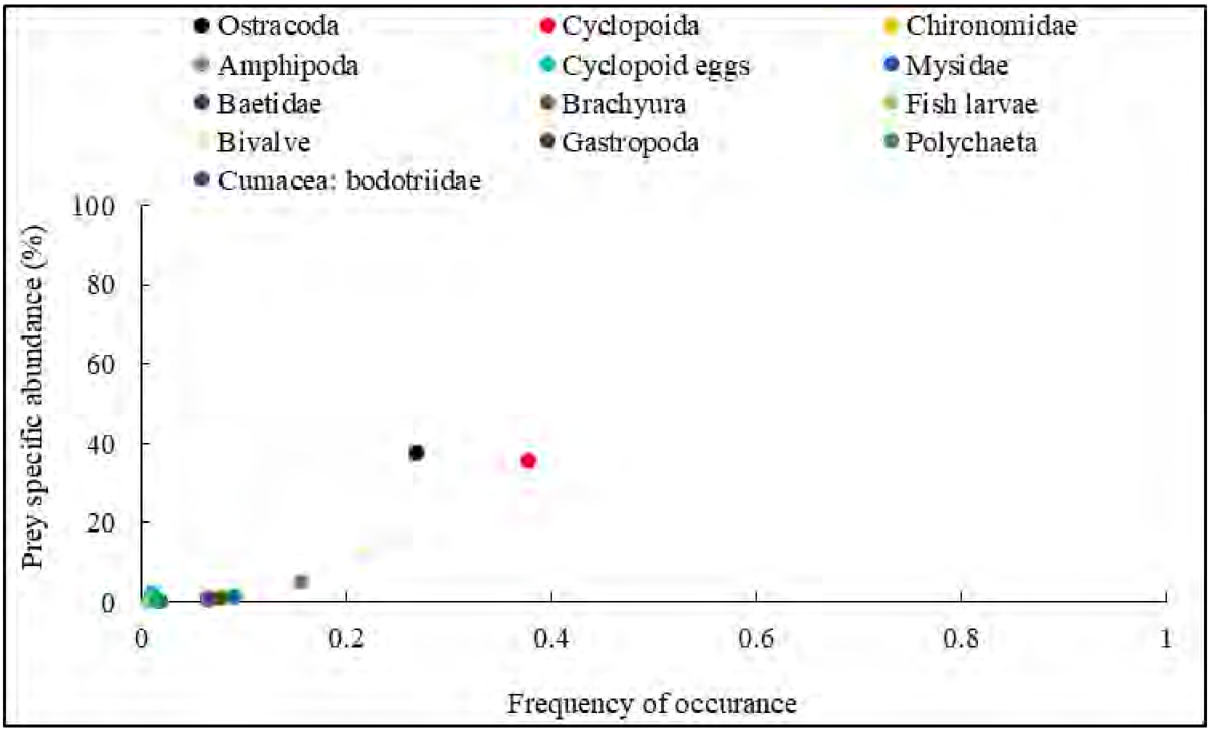


Figure 5.4: Feeding strategy showing prey selection by *Psammogobius knysnaensis* in the Sundays Estuary, Eastern Cape, South Africa

Annual changes in diet

Annual changes in the diets of *P. knysnaensis* were observed in both small (juvenile 20–39 mm TL) and large (adult > 40 mm TL) size individuals ($\chi^2 = 279.6$, df = 36, $p < 0.05$; Figure 5.5). During September/October 2018, cyclopoids (IRI = 65.3%) had a very high contribution to the diet followed by bivalves (IRI = 24.49%) and ostracods (IRI = 6.98%). In the adult size classes, bivalves comprised a large proportion of the diet (IRI = 81.35%) and cyclopoid consumption was low (IRI = 5.70%). In 2019, juveniles consumed more ostracods (IRI = 55.80%) and cyclopoids (IRI = 36.54%). In the adult size classes, the consumption of ostracods (IRI = 34.50%) decreased slightly and that of cyclopoids (IRI = 41.96%) and bivalves (IRI = 10.52%) slightly increased.

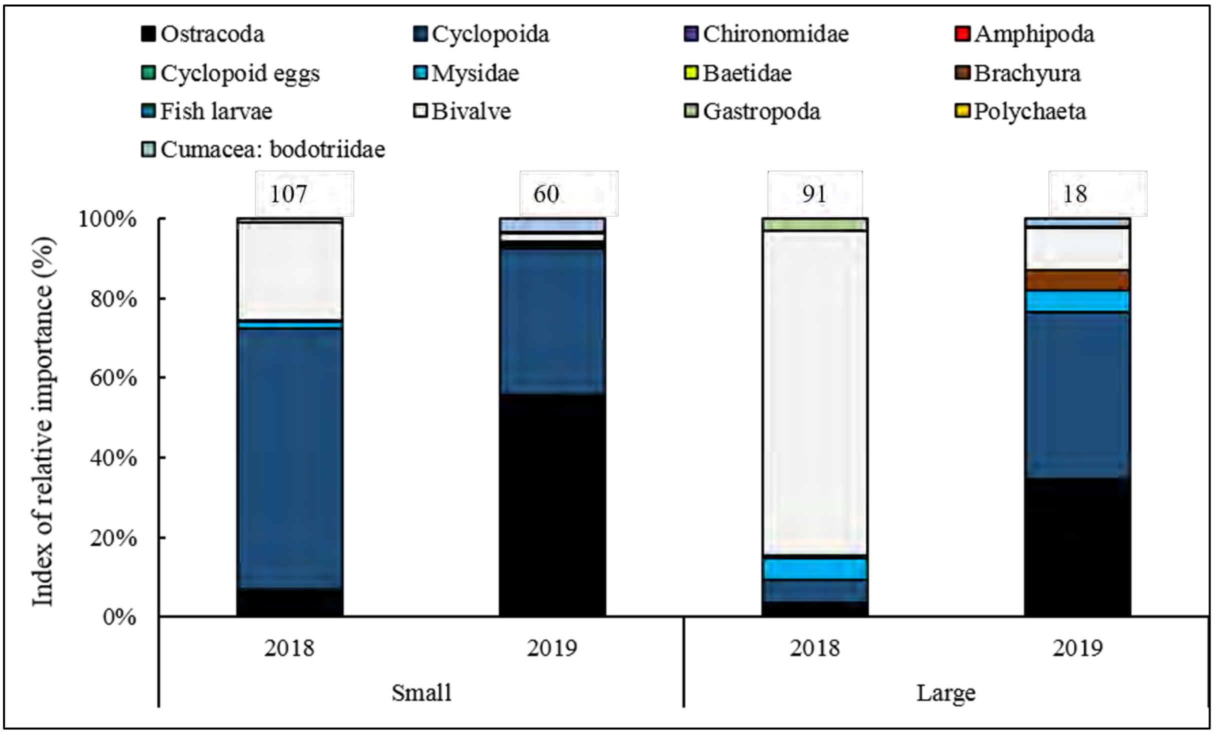


Figure 5.5: Seasonal comparisons of percentage index of relative importance (%IRI) of prey items found in the stomachs of *Psammogobius knysnaensis* in spring 2018 and winter 2019 in the Sundays Estuary, Eastern Cape, South Africa. Small = juveniles (20–39 mm TL) and large = adults (> 40 mm TL)

Caffrogobius gilchristi

<u>*Diet and ontogenetic shifts*</u>

A total of 154 stomachs of *C. gilchristi* were analysed. Of all the stomachs analysed, only one was empty. A total of 12 prey taxa were identified in the diet of *C. gilchristi* (Figure 5.6 and Table 5.1), with Cyclopoida having the highest contribution (20–49 mm = > 60%; > 49 mm = > 30 %) to this species' diet both in terms of %IRI and %N.

Prey consumption changed significantly with fish size (χ^2 = 165.4, df = 33, $p < 0.05$). Cyclopoida consumption decreased from the 20–29 (IRI = 87.84%), 30–39 (IRI = 97.34%) and 40–49 mm (IRI = 82.79%) size classes to the 50–59 mm size class (IRI = 31.4). The largest size class (50–59 mm) consumed the highest diversity of prey, with cyclopoida (IRI = 31.39%), mysidae (IRI = 26.16%), amphipoda (IRI = 14.83%), brachyura (IRI = 11.70%) and gastropoda (11.09%) dominating the diet. Mysidae were only recorded in the stomachs of individuals greater than 40 mm TL. An ontogenetic shift in diet occurred from 50 mm TL, when cyclopods comprised less than 50% of the diet (<50% IRI) (Figure 5.6). Gastropoda were only recorded in the stomachs of individuals greater than 30 mm TL, and their proportion in the diet increased with fish size. Brachyura, although consumed by all size classes, were recorded in the greatest numbers in the smallest (20–29 mm TL) (IRI = 6.80%) and largest size classes (50–59 mm TL) (IRI = 11.70%). Ostracods, although also consumed by all size classes, comprised a relatively small proportion of the diet in all size classes (3.1–4.4 %IRI).

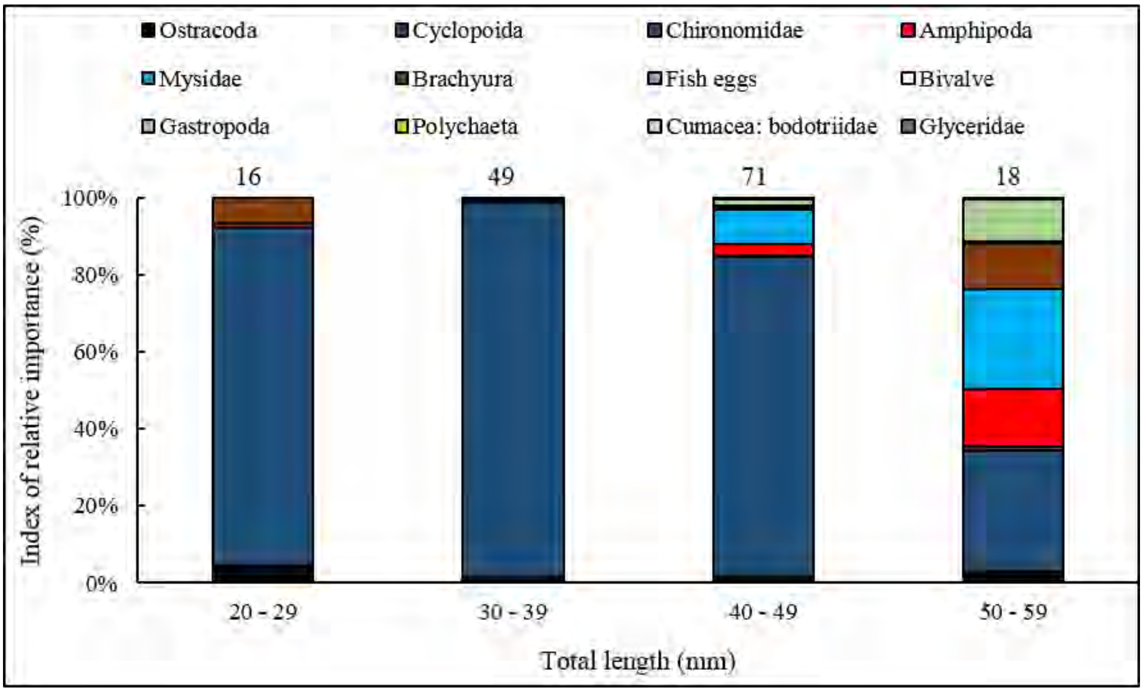


Figure 5.6: Percentage index of relative importance (%IRI) of prey items found in the stomachs of different size classes of *Caffrogobius gilchristi* from combined years in the Sundays Estuary, Eastern Cape, South Africa. Numbers above bars represent samples size

The largest size class (50–59 mm) had the highest niche breadth, comprising nine prey taxa (Figure 5.7). These were cyclopoida, chironomidae, amphipoda, mysidae, brachyuran, bivalve, gastropoda and glyceridae. The smallest size class (20–29 mm) had the second largest niche breadth, comprising ostracoda, cyclopoida, chironomidae, amphipoda and brachyura.

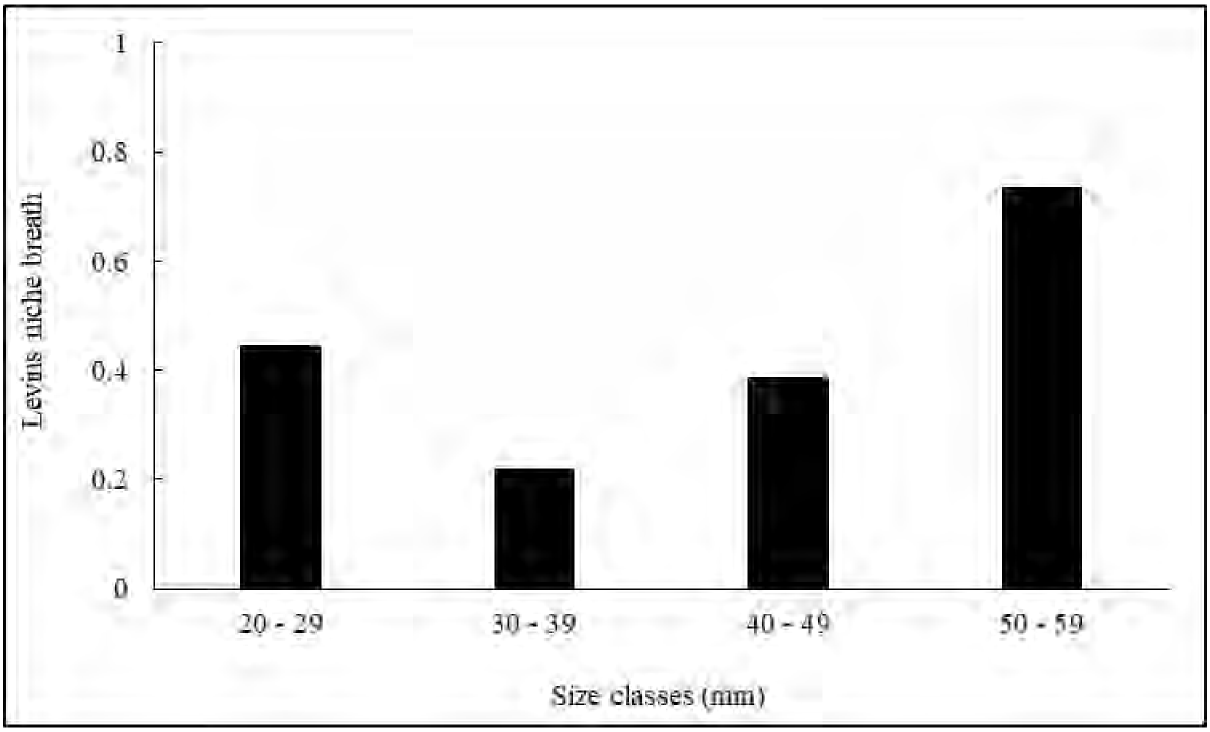


Figure 5.7: Dietary Levins niche breadth of *Caffrogobius gilchristi* size classes collected from the Sundays Estuary, Eastern Cape South Africa

Feeding strategy

Caffrogobius gilchristi was a specialist feeder on cyclopoids (Figure 5.8). This prey group dominated the diet of *C. gilchristi* more than the other prey groups. This species was an opportunistic feeder on ostracoda, chironomidae, amphipoda, cyclopoid eggs, mysidae, baetidae, brachyuran, fish eggs, fish larvae, bivalve, gastropoda, polychaeta, cumacea: bodotriidae, glyceridae and calanoids. It was referred as an opportunist feeder on these prey groups because they all had a very low contribution to their diet.

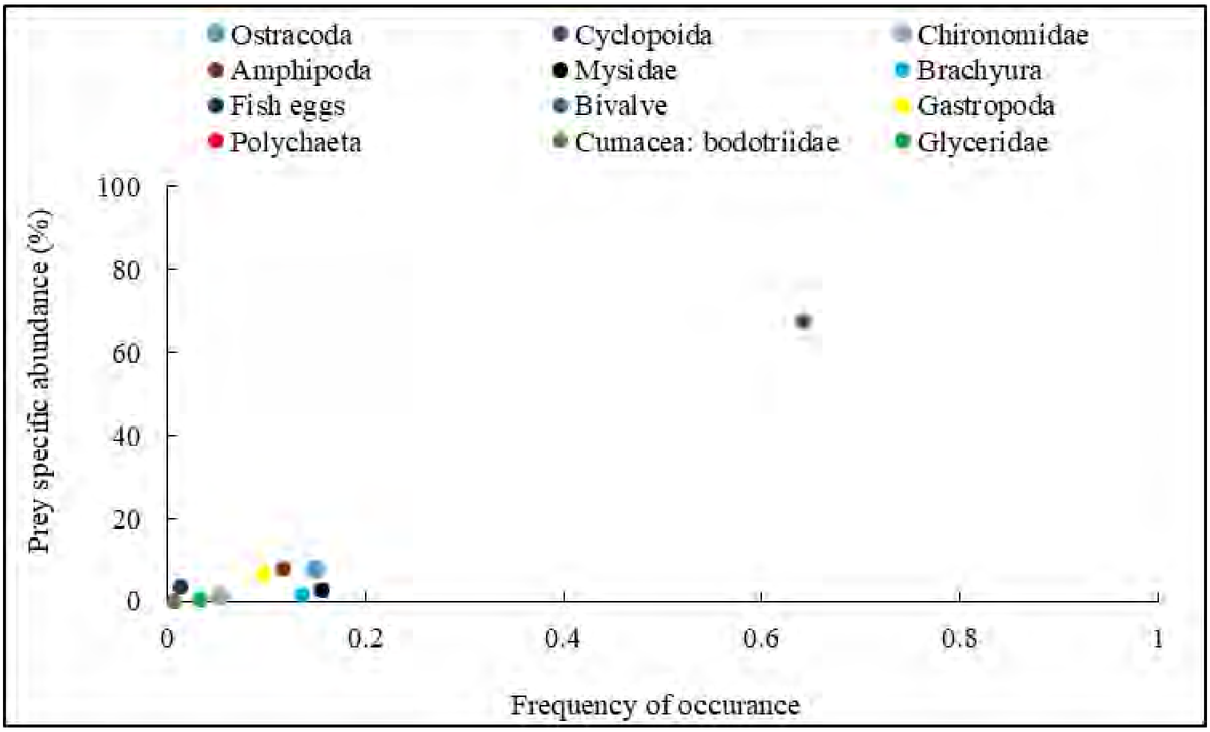


Figure 5.8: Feeding strategy showing prey selection by *Caffrogobius gilchristi* in the Sundays Estuary, Eastern Cape, South Africa

Annual changes in diet

Annual changes were particularly evident in the diet of *C. gilchristi* (Figure 5.9) ($\chi^2 = 70.3$, df = 33, $p < 0.05$). For large (adult > 45 mm) size individuals, the consumption of cyclopoids (IRI = 5.5% and IRI = 58.6%) increased from 2018 to 2019, while the consumption of mysids decreased substantially from 2018 to 2019 (IRI = 47.9 and IRI = 6%). The consumption of crabs (IRI = 7.2 and 22.5%) in the adult size classes also increased between 2018 and 2019. Amphipoda (IRI = 21.6%) and gastropoda (IRI = 15.7%) were only recorded in the diet of adult individuals in 2018.

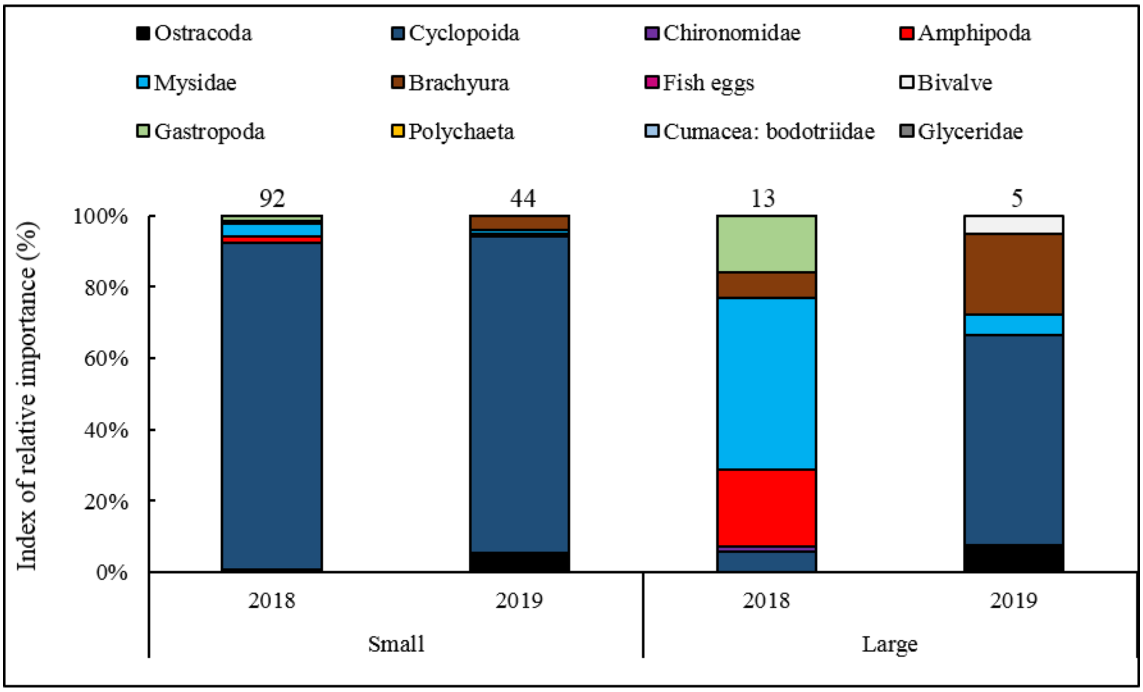


Figure 5.9: Seasonal comparisons of percentage index of relative importance (%IRI) of prey items found in the stomachs of juvenile (20–49 mm) and adult (> 50 mm) *Caffrogobius gilchristi* in spring 2018 and winter 2019 in the Sundays Estuary, Eastern Cape, South Africa

Glossogobius callidus

Diet and ontogenetic shifts

A total of 146 stomachs of *G. callidus* were analysed. Of the stomachs analysed only one was empty. A total of 10 prey taxa were identified in the diet of this species (Figure 5.10 and Table 5.1), with amphipods, chironomids and cyclopoids being dominant in the diet of this species. The consumption of these prey groups significantly changed with fish size ($\chi^2 = 229$, df = 45, $p < 0.05$). Freshwater prey (amphipods and chironomids) consumption by *G. callidus* increased with fish size, while cyclopoid consumption decreased with fish size. Cyclopoids dominated the diet of individuals of less than 50 mm. Very few cyclopoids were consumed by the 60–69 and > 69 mm size classes (IRI = 5.08 and 1.57%), with amphipods and chironomids being dominant. An ontogenetic dietary shift was observed at 40 mm, as above this length the consumption of cyclopoids was less than 50% (IRI), with amphipod, chironomids and baetidae increasing in importance. Calanoids (IRI = 2.42%) and mysid (IRI = 3.32%) consumption was only noticeable in the diet of the largest size class (> 69 mm).

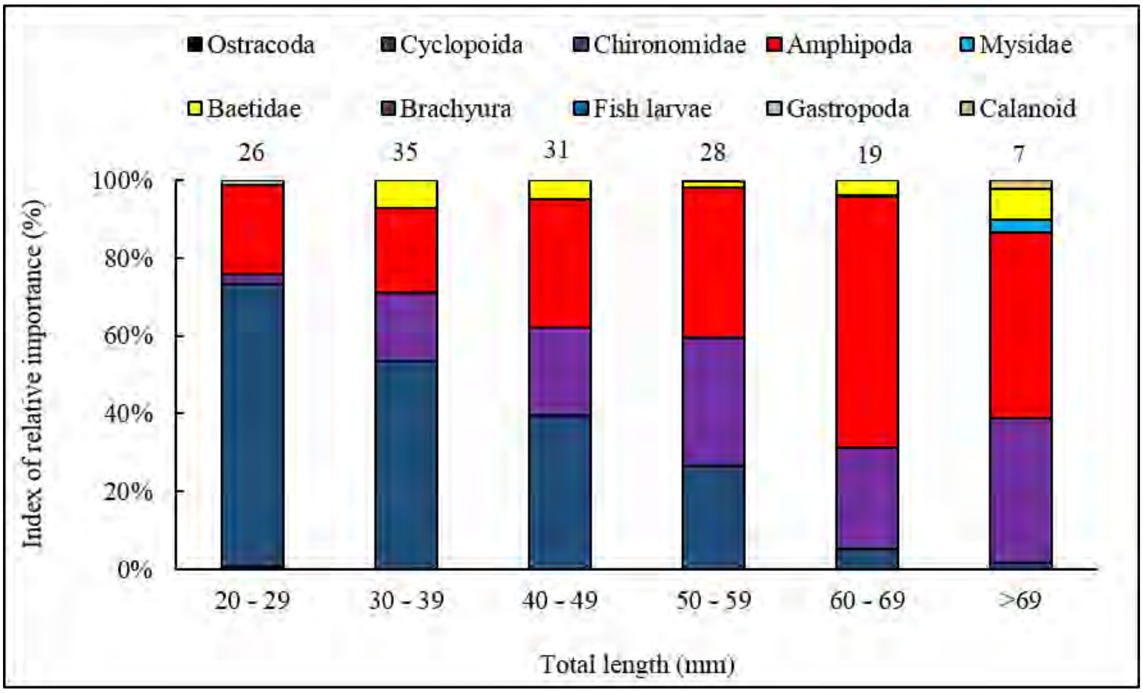


Figure 5.10: Combined years percentage index of relative importance (%IRI) of prey items found in the stomachs of different size classes of *Glossogobius callidus* in the Sundays Estuary, Eastern Cape, South Africa. Numbers above bars represent sample size

Individuals of 30–39 mm had the highest niche breadth, with their diet comprising cyclopoida, chironomidae, amphipoda and baetidae. The largest size class (> 60 mm) had the second highest niche breadth, comprising cyclopoida, chironomidae, amphipoda, mysidae, baetidae and calanoid. The consumption of ostracods was only observed in individuals of less than 60 mm. Mysids were consumed by individuals greater than 50 mm (Figure 5.11).

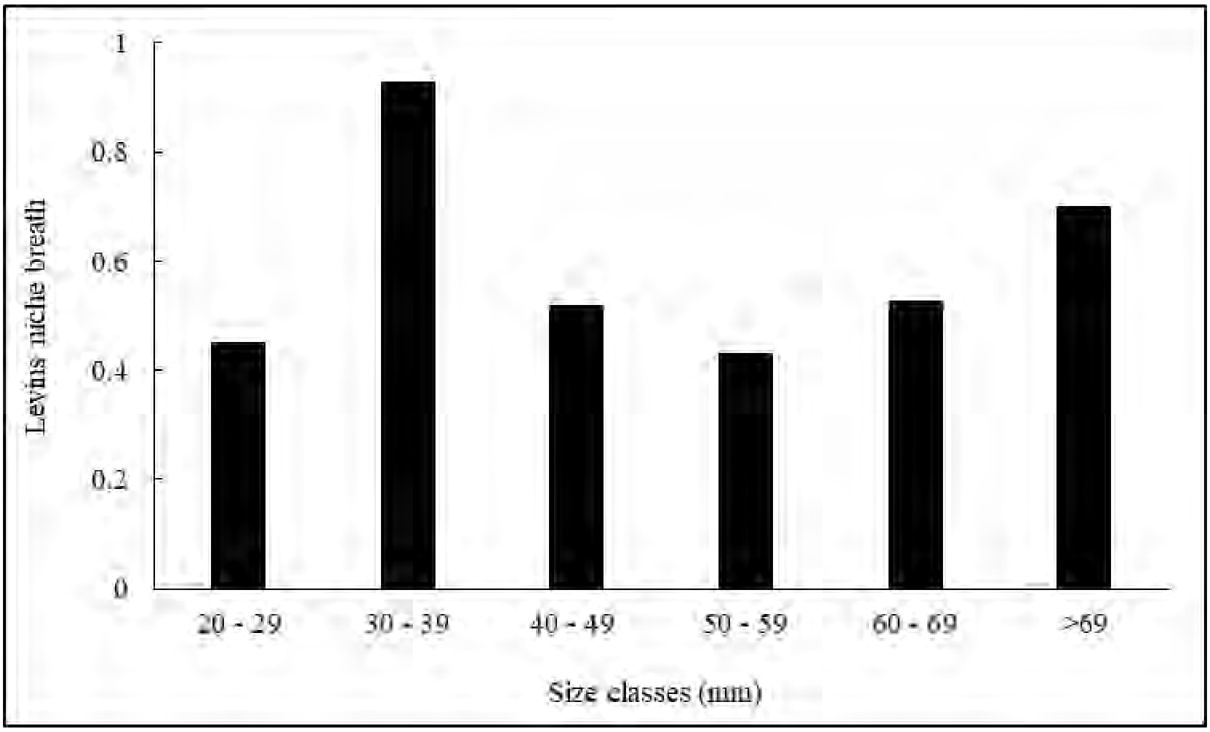


Figure 5.11: Dietary Levins niche breadth of *Glossogobius callidus* size classes collected from the Sundays Estuary, Eastern Cape South Africa

Feeding strategy

Glossogobius callidus was a generalist feeder on cyclopoids, amphipods and chironomids. It was an opportunistic feeder on ostracods, baetidae, brachyura, fish larvae, gastropods, mysids and calanoids (Figure 5.12).

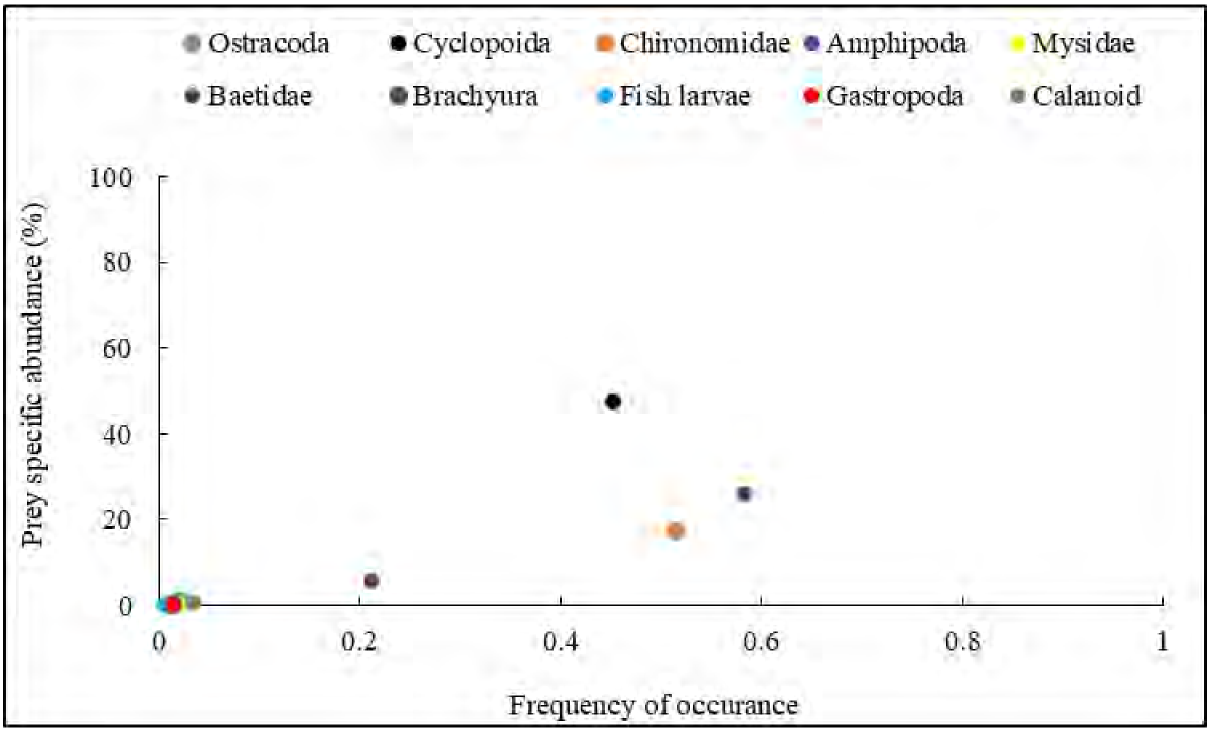


Figure 5.12: Feeding strategy showing prey selection by *Glossogobius callidus* in the Sundays Estuary, Eastern Cape, South Africa

Annual changes in diet

Significant annual changes ($\chi^2 = 245.2$, df = 27, $p < 0.05$) in the diet of *G. callidus* were observed between 2018 and 2019, more especially in the smallest size class (20–39 mm) (Figure 5.13). Amphipods (IRI = 56.4%) dominated the diet of small sized individuals in spring 2018. Cyclopoids (IRI = 34.7%) and chironomids (IRI = 5.1%) also occurred in high numbers in spring 2018. In winter 2019, there was lower consumption of amphipods (IRI = 13.1%), with cyclopoids (IRI = 60.14%) dominating the diet of small individuals. The diet of large (> 40 mm TL) individuals in the spring 2018 survey was more similar to that of small sized individuals. Amphipods (IRI = 78.8%) and chironomids (IRI = 18.2%) dominated the diet, but the consumption of cyclopoids (IRI = 2.4%) was reduced. In 2019, cyclopoid consumption (IRI = 35.1%) was reduced and the consumption of baetidae (IRI = 9.8%), amphipods (IRI = 20.1%) and chironomids (IRI = 34.5%) was high in the large size individuals.

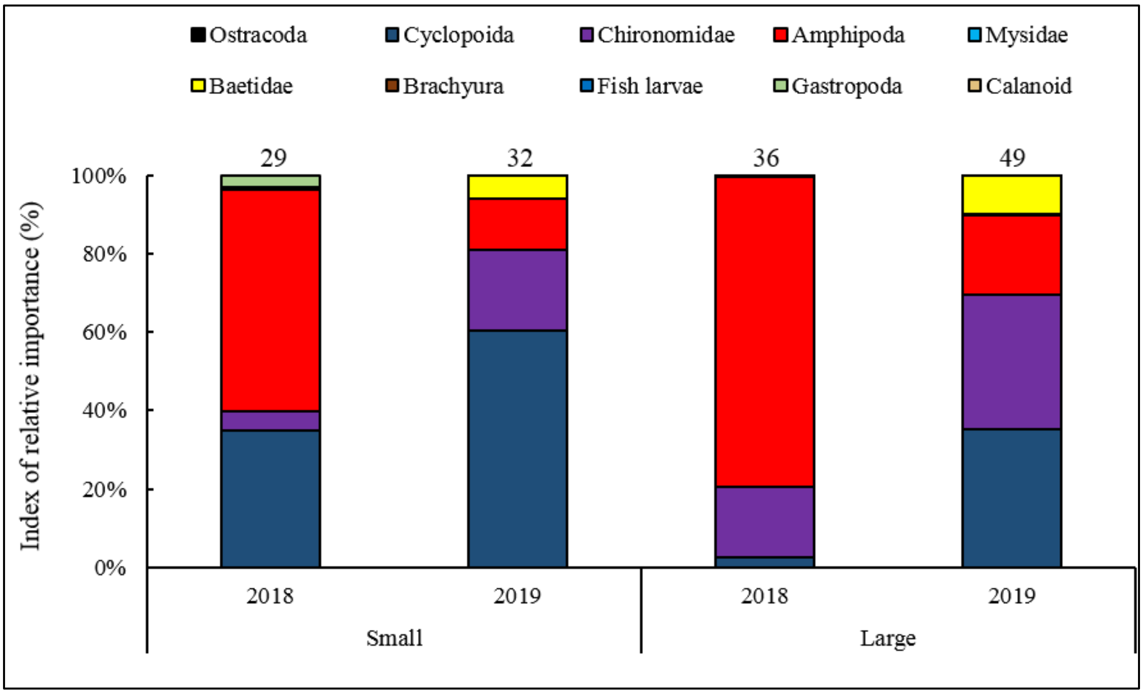


Figure 5.13: Seasonal comparisons of percentage index of relative importance (%IRI) of prey items found in the stomachs of small (20–39 mm) and large (40–59 mm) *Glossogobius callidus* in spring 2018 and winter 2019 in the Sundays Estuary, Eastern Cape, South Africa. Small = juveniles and large = adults. Numbers above bars indicate sample size.

Interspecies comparisons

Lower reaches

Interspecies comparisons were only done between species where they co-occurred in the estuary. *Psammogobius knysnaensis* and *C. gilchristi* were caught together in the lower reaches of the estuary. Cyclopoids were common in the diet of both species during juvenile sizes (< 40 and < 50 mm respectively) (Figure 5.14). Among the juvenile size classes, *C. gilchristi* specialised in cyclopoids, which constituted IRI = 91.6% of their diet. In the diet of juvenile sizes (< 40 mm) of *P. knysnaensis,* both cyclopoids (IRI = 49.1%) and ostracods (IRI = 40.0%) were important in the diet. Adult sized *C. gilchristi* individuals were generalist feeders, feeding on mysids, (37.2% IRI) cyclopoids (22.8% IRI), amphipods (12.4% IRI), brachyura (15.1% IRI) and gastropods (9.1% IRI). The consumption of cyclopoids (IRI = 18.9% IRI) was low for adult size *P. knysnaensis* but consumption of bivalves (IRI = 57.4% IRI) was very high.

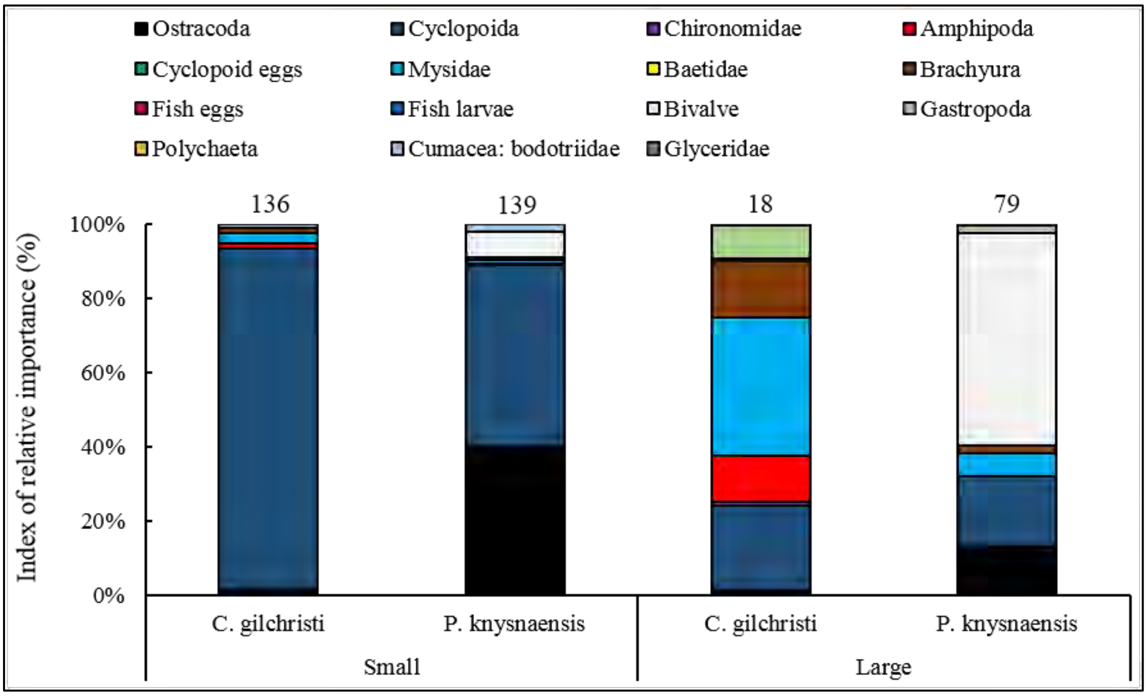


Figure 5.14: dietary comparisons of percentage index of relative importance (%IRI) of prey items found in the stomachs of small (20–39 mm) and large (40–59 mm) *Psammogobius knysnaensis* and *Caffrogobius gilchristi* in spring 2018 and winter 2019 in the Sundays Estuary, Eastern Cape, South Africa. Numbers above bars indicate sample size.

Psammogobius knysnaensis juvenile and adult sized individuals had more similar diets than both small and large sized *C. gilchristi* individuals (Figure 5.15).

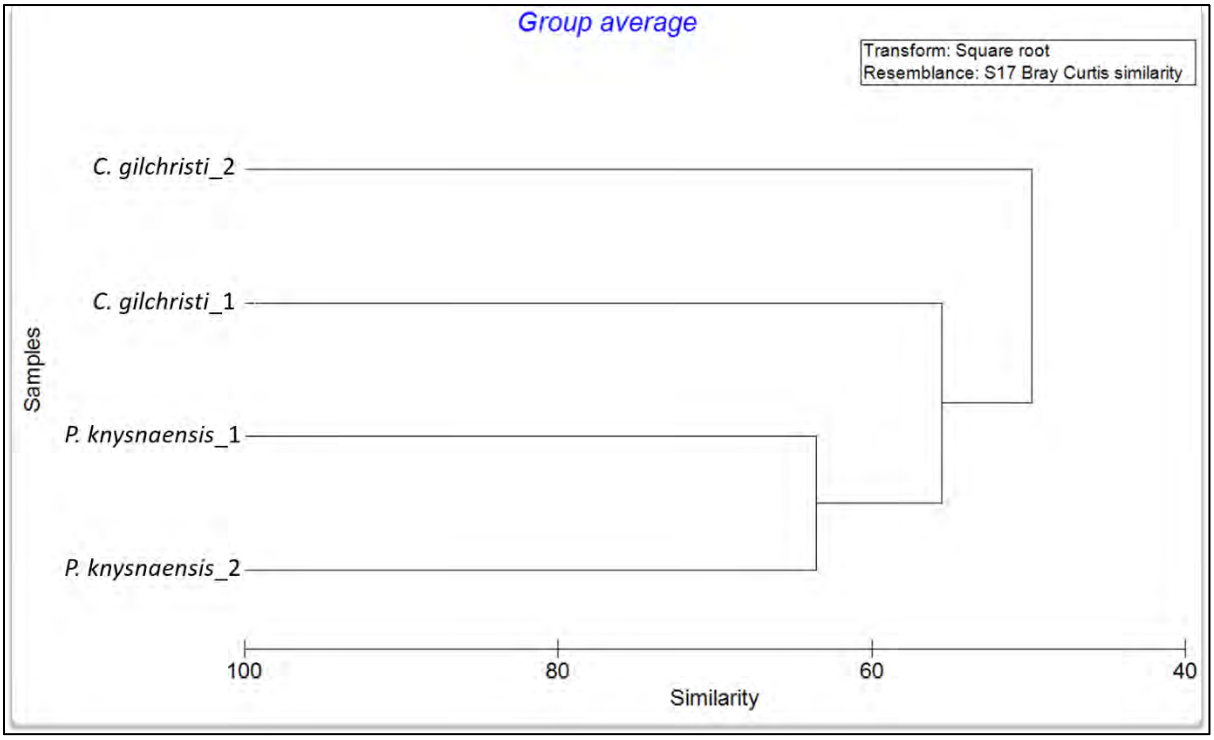


Figure 5.15: Cluster analysis showing dietary similarities between small (20–39 mm) and large (40–49 mm) size *P. knysnaensis* and *C. gilchristi* in the Sundays Estuary, Eastern Cape, South Africa. _1 = small size and _2 = large size.

<u>*Upper reaches*</u>

In the upper reaches of the estuary *G. callidus* and *P. knysnaensis* were caught together. Juvenile size individuals of *G. callidus* (20–39 mm) and *P. knysnaensis* (20–39 mm) preyed extensively on cyclopoids (IRI = 61.1 and 69.1%) and amphipods (IRI = 24.6 and 12.0%), which were abundant in the diet of both species (Figure 5.16). *Glossogobius callidus* also specialised in chironomids (IRI = 10.7%), while *P. knysnaensis* specialised in consuming ostracods (IRI = 16.0%). When comparing adult size individuals (*G. callidus* = 20–39 mm) of *G. callidus*, the consumption of amphipods (IRI = 46.3%) and chironomids (IRI = 30.1%) increased, while that of cyclopoids (IRI = 19.3%) decreased. The consumption of amphipods (IRI = 62.0%) also increased in the adult size classes *P. knysnaensis* (40–59 mm) and that of cyclopoids (IRI = 28.4%) decreased.

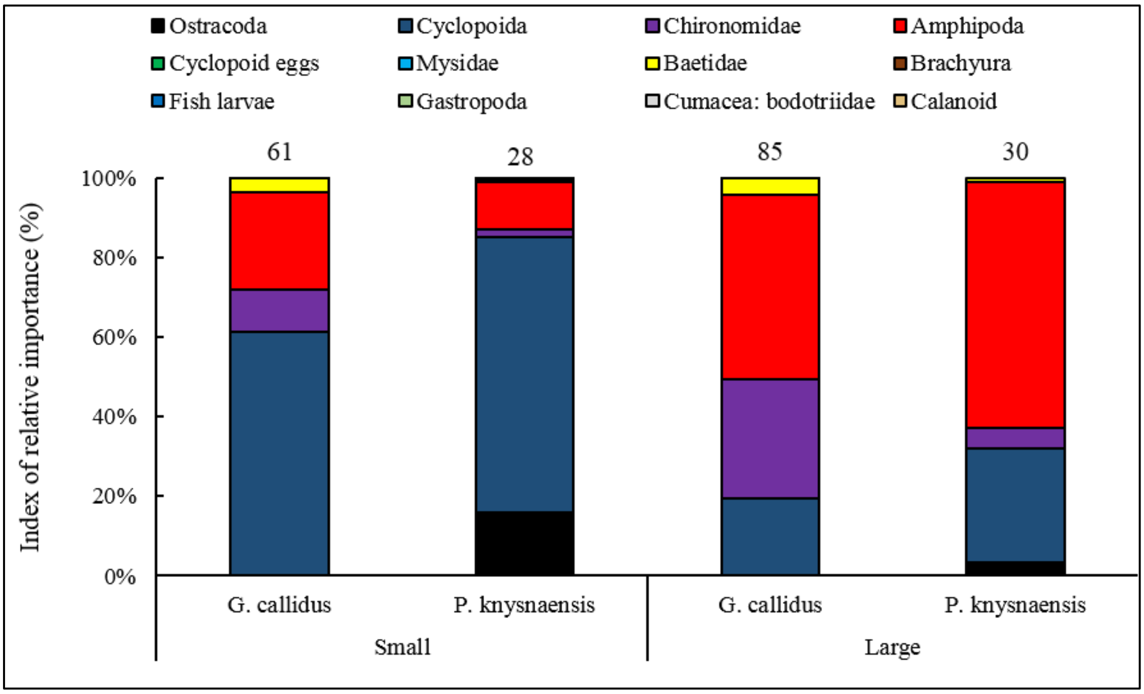


Figure 5.16: Dietary comparisons of percentage index of relative importance (%IRI) of prey items found in the stomachs of small and large *Psammogobius knysnaensis* and *Glossogobius callidus* in spring 2018 and winter 2019 in the Sundays Estuary, Eastern Cape, South Africa. *Psammogobius knysnaensis* small and large individuals were represented by 20–39 mm and 40–59 mm individuals and those of *G. callidus* were represented by 20–59 and 60 - > 69 mm individuals. Numbers above bars indicate sample size.

Psammogobius knysnaensis and *G. callidus* large individuals were more similar to each other (Figure 5.17). Chironomids, ostracods and amphipods contributed 36.11, 19.87 and 12.01% respectively to this similarity. Small *P. knysnaensis* and *G. callidus* differed.

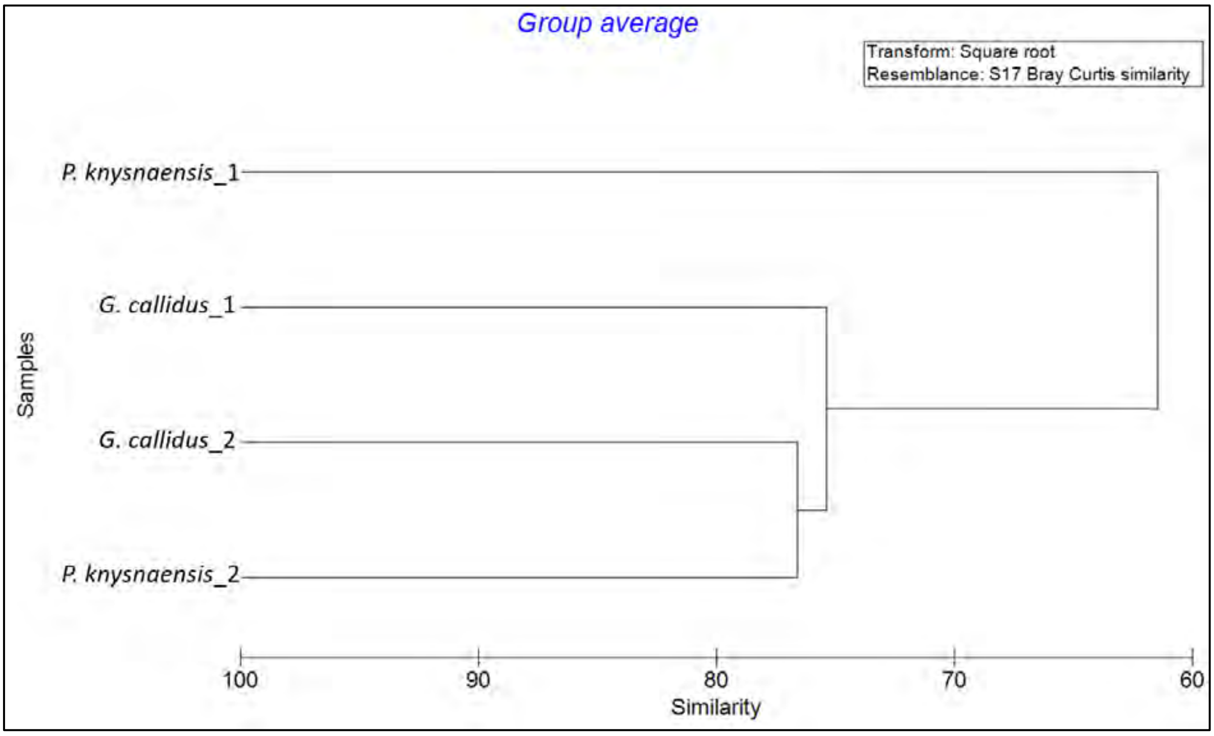


Figure 5.17: Cluster analysis showing dietary similarities between small and large size *P. knysnaensis* and *C. gilchristi* in the Sundays Estuary, Eastern Cape, South Africa. *Psammogobius knysnaensis* small and large individuals were represented by 20–39 mm and 40–59 mm. *Glossogobius callidus* small and large individuals were represented by 20–59 and 60–> 69 mm. _1 = small size and _2 = large size.

Discussion

This component of the study focused on describing the diet, food resource partitioning and feeding behaviour of *Psammogobius knysnaensis, Caffrogobius gilchristi* and *Glossogobius callidus* in the Sundays Estuary. All three species were carnivorous feeders, feeding on benthic invertebrates and zooplankton. Even though morphological prediction from Chapter 4 held that *P. knysnaensis* and *C. gilchristi* have different diets, the opposite was observed in this study among the small sizes. Niche segregation was observed only in the large size individuals. Dietary overlap between *P. knysnaensis* and *G. callidus* was observed. These two species had a more similar dietary niche among both small and large sizes than predicted in the morphological analysis.

As with many fish species which change their diet from feeding on small size less energy gain prey to large prey with high energy gain as they develop (Gaughan and Potter, 1987; Choi and Suk, 2012; Guo et al., 2014), this was true in our study. In general, during adult stages, fishes are known to select high energy gain food (Horn, 1983). In this study, cyclopoids and ostracods were common in the diet of small size individuals of all three species. Bivalves, amphipods, chironomids, baetidae, mysids and gastropods were common in the diet of large size individuals. This dietary similarity among these species could be the result of overlapping feeding morphology, as observed in Chapter 4. This dietary change with size is common in fishes (Pereira et al., 2015). In a study by Gkenas et al. (2012), gobies shifted their diets from small, soft-shelled prey (microcrustaceans and chironomidae) to large size prey such as gastropods, cladocerans and insects. Bennett et al. (1989) also found that juvenile *P. knysnaensis* specialised in consuming ostracods and copepods, while adults specialised in the consumption of amphipods and decapods. Similarly, specialising in cyclopoids during juvenile stages is common in the *Caffrogobius* species. For example, in the Palmiet Estuary, juvenile *Caffrogobius multifasciatus* were found to be specialist feeders on cyclopoids (Bennett, 1989). In other related species such as *Caffrogobius caffer*, copepods were recorded in the diet of juvenile fish, and molluscs were only recorded in adults (Butler, 1980). The diet richness (number of prey taxa) also increased with size for *Psammogobius knysnaensis* and *C. gilchristi.*

The diet richness of *G. callidus* was generally high in all size classes, with this species being a generalist feeder on cyclopoids, amphipods and chironomids. Previous studies have also shown that this species is a generalist feeder (Vumazonke, 2008; Wasserman, 2012; Mofu et al., 2019). As with other gobies (St John et al., 1989; Mesa et al., 2008; Vumazonke, 2008; Wasserman, 2012), all three species were shown to have a high preference for cyclopoids.

Psammogobius knysnaensis' diet changed with prey availability. During the 2018 sampling, *P. knysnaensis* was a generalist feeder shifting from specialising in consuming cyclopoids, to bivalves with an increase in fish size. During the 2019 sampling, *P. knysnaensis* in the lower section of the estuary was also a generalist feeder but mostly consumed small soft prey like ostracods and cyclopoids. It continued specialising in the consumption of the same prey among the large sizes, but the consumption of bivalves, brachyura and mysids also increased. The increase of the incorporation of hard prey among the large sizes could be promoted by their long guts, which allows for longer digestion time. *Caffrogobius gilchristi* generally specialised in consumption of cyclopoids in both seasons. In spring 2018, *C. gilchristi* shifted from specialist (cyclopoids) to generalist feeding. During the winter period, it also specialised in cyclopoids, but increased its consumption of brachyura and mysids in the large size class individuals. Differences were also observed in the *G. callidus* diet between sampling occasions.

Periodic changes in food availability in feeding habitats can result in change in fish diet in some species (Layman and Silliman, 2002; Contente et al., 2012; Gkenas et al., 2012; Hajji et al., 2012; Kovacic, 2012a), whereas in other species this does not occur (Feyrer et al., 2003). In the Danube River, *Apollonia melanostoma* diets changed with seasons, but that of the closely related *Neogobius melanostomus* was not affected by seasonal changes (Borza et al., 2009). In this study, fish diets changed across the sampling occasions (different month and year) and this may be related to prey availability.

Prey availability in the feeding environments is known to affect fish diet (Carle and Hastings, 1982). Darter Goby *Gobionellus boleosoma* (Jordan and Gilbert, 1882) diet reflects the abundance of its prey items in the environment (Carle and Hastings, 1982). Copepods, ostracods and nematodes, abundant in their diet, were also abundant in the environment (Carle and Hastings, 1982). *Gymnogobius isaza* (Tanaka, 1916) diet in Lake Biwa, Japan, was also influenced by prey availability (Briones et al., 2012). According to Jerling and Wooldridge (1995) and based on the results of Chapter 2, copepods are widely distributed along the length of the Sundays Estuary, and this may explain their high consumption by *P. knysnaensis* and *C. gilchristi* in both sampling periods. These studies also showed that mysids are abundant in the lower section of this estuary, and this explains why they were common in the diet of large size individuals of these two species (Jerling and Wooldridge, 1995; Chapter 2). Although the diet of *G. callidus* changed with sampling occasions, its composition (chironomids, baetidae and amphipods) reflected the prey items present in this region of the estuary (Jerling and Wooldridge, 1995; Chapter 2).

As observed in this study, the diet shift of these species according to fish size, resulted in changes in the dietary overlap between size classes. Fishes tend to have high dietary overlap among the small sizes, but feed on different prey as they develop. For example, *Rhinogobius cliffordpopei* and *Rhinogobius giurinus* have a higher dietary overlap among small sizes than large sizes (Guo et al., 2014). In the lower reaches of the estuary, cyclopoids were common in the diet of small co-occurring *P. knysnaensis* and *C. gilchristi*, but the two species preferred different diets among the large sizes. However, the dominance of cyclopoids in the diet of gobies is a common phenomenon within this family (St John et al., 1989; Mesa et al., 2008) and this may be due to their high abundance in the feeding environments (Chapter 2). In the upper reaches, *P. knysnaensis* and *G. callidus* both had high consumption of cyclopoids and amphipods among the small size classes, with only amphipods common in the diet of the large sizes.

When two species feed in different habitats, they can feed on the same prey without competing (Su et al., 2016). In a study by Pimentel and Joyeux (2010) in the Piraquê-açú and Piraquê-mirim Rivers, three *Lutjanus* species had overlapping diets but the three species partitioned their food resources by feeding in different environments. In the Mediterranean infralittoral rocky habitats, two *Diplodus* species had overlapping diets but fed in different habitats (Sala and Ballesteros, 1997). Similarly, in this study, cyclopoids were common in the diet of all three species, but it cannot be concluded that they compete for this resource as different species are abundant in different sections of the estuary (Richardson et al., 2006; Bailey and James, 2013; Nodo et al., 2018). Although abundant in the lower section of estuaries, *P. knysnaensis* is often more abundant in the sandy mouth region of estuaries, whereas *C. gilchristi* is more abundant in the muddy habitats of the lower and middle reaches of estuaries (Richardson et al., 2006; Bailey and James, 2013; Nodo et al., 2018). On the other hand, *G. callidus* is known to be abundant in the middle and upper reaches, and this may explain how the three species are all able to feed on cyclopoids.

Conclusion

Ontogenetic dietary shifts were observed in all three species. Ostracods and cyclopoids were preferred by small size fishes, while bivalves, amphipods, chironomids, baetidae, mysids and gastropods were common in the diet of large size individuals. Temporal changes in diet were also observed between the three species, probably driven by periodic changes in prey availability. Dietary overlap decreased with fish size and the larger individuals specialised in different prey as they developed. As predicted by their morphology, large size *P. knysnaensis* and *C. gilchristi* specialised on different diets while that of *P. knysnaensis* and *G. callidus* highly overlapped in the upper section of the estuary. As these findings indicate, the potential for resource competition between juveniles (smaller stages) (particularly for *G. callidus* and *P. knysnaensis*), changes according to the abundance and distribution of these three species in the estuary, could be investigated to provide explanation for their coexistance.

CHAPTER SIX

Abundance and habitat partitioning of *Psammogobius knysnaensis, Caffrogobius gilchristi* and *Glossogobius callidus* in the Sundays Estuary

Introduction

The distribution of estuarine fishes is determined by biotic factors (such as competition, food availability), abiotic factors (such as salinity, temperature, substrate) and landscape factors (such as habitat size and availability) (Malavasi et al., 2005). Studies on the distribution of fish species and the factors that influence these patterns are important in understanding community structure (Wilkins and Myers, 1995) and the co-existence of morphologically similar species within estuaries (Leitão et al., 2006). Fishes can partition their habitat by size or species, with different sized individuals of the same species or different species living in different habitats (Macpherson, 1981; Malavasi et al., 2005; Leitão et al., 2006). Habitat partitioning by coexisting species minimises the strength of interspecific competition between similar species feeding on the same resources (Macpherson 1981; Humphries and Potter 1993; Malavasi et al., 2005; Leitão et al., 2006; Horinouchi, 2008).

Gobies, which are an important group within estuaries in terms of abundance and species richness, share a similar morphology due to their adaptation to a benthic life (Malavasi et al., 2005). Food and habitat partitioning (spatial segregation) have been identified as key to allowing for the co-existence of these closely related species in tropical and temperate estuaries and coastal waters (Malavasi et al., 2005). In two coral-dwelling gobies, the broad-barred goby *Gobiodon histrio* and blue-spotted coral-goby *Gobiodon erythrospilus*, juveniles have been observed to compete for space (known as the lottery model), while adults partition their habitats, suggesting that resource partitioning increases with ontogeny (Pereira et al., 2015).

Sediment type has been identified as a key driver in the distribution of goby species in soft-bottom habitats in South African estuaries. In both permanently open and temporarily open/closed estuaries the Knysna sand goby *Psammogobius knysnaensis* is particularly abundant in sandy areas in the mouth region (Cowley and Whitfield, 2001; Richardson et al., 2006; Bailey and James, 2013; Nodo et al., 2018). Within the lower reaches *P. knysnaensis* uses firm objects (such as rocks) for nesting, which may also explain its preference for the mouth region of permanently open estuaries, which typically have rocky outcrops at the mouth (Wasserman et al., 2017). The river goby *Glossogobius callidus* (Smith, 1937), although distributed throughout estuaries, is typically more abundant in the muddy upper reaches (Cowley and Whitfield, 2001; Richardson et al., 2006; Nodo et al., 2017). *Caffrogobius gilchristi* tends to occur in the lower and middle reaches of permanently open estuaries (Nodo et al., 2018; James et al., 2020).

Prey type and availability is another key factor that influences fish distribution and abundance. The survival, growth and reproduction of fishes depends on their energy investment from food, and this explains why they prefer areas with high prey availability (Pecquerie et al., 2009; McBride et al., 2015). For specialist feeding species, prey availability greatly influences species distribution. In a study by Griffiths (1997) conducted in the Great Fish Estuary, dusky kob *Argyrosomus japonicus* (Temminck; Schlegel, 1843) distribution was influenced by prey availability, and dusky kob were abundant in areas where their prey was abundant.

In this chapter, the driving factors behind habitat partitioning in three selected Gobiidae species were investigated in the Sundays Estuary. This was accomplished by determining (i) which environmental variables define different channel habitats in the estuary and (ii) how the distribution and abundance of the different species is associated with biotic and abiotic factors in the Sundays Estuary. It was hypothesised that *P. knysnaensis* and *G. callidus* would occupy different habitats, as they have highly overlapping diets, particularly in the juvenile stages (Chapter 5).

Materials and methods

Field sampling

Sampling was undertaken three times a season across nine sites from March 2018 to September 2019 (Figure 2.1). For fish sampling, a three-meter-long beam-trawl net constructed from 14 mm mesh size was used. The net was 1.5 m wide and conical, tapering to 0.5 m in the cod end, with 6.5 mm knotless nylon mesh at the end of the net (Nodo et al., 2018). During sampling the net was towed 20 m behind the boat at a speed of ~2 knots for three minutes. One trawl was done at each site, covering a distance of approximately 200 m. After each trawl fish were identified and sorted, with the three target species measured (mm TL) and released back to the water alive. Only the three study species were measured and identified, all other species were released immediately.

At each site the physicochemical parameters (temperature, pH, salinity, depth, turbidity and dissolved oxygen) were recorded using a YSI multi-parameter probe. All measurements were taken at two water levels (bottom and surface) but only bottom measurements were used for this analysis. Sediments (%mud and %sand) were also collected from each site with details provided in Chapter 2.

Sediments were collected at each site in March 2020 using a cone dredge for sediment particle size analysis. Collected sediments were stored in plastic containers in a cooler box on ice for later analysis in the laboratory (detailed in Chapter 2).

Data analysis

Mean catch per unit effort (CPUE), calculated as the number of fish/haul in each season, was used as an index of fish abundance. All three species were divided into small and large sized individuals based on 50% maturity and changes in diet with ontology. For *P. knysnaensis* and *G. callidus,* small sized individuals (juveniles) were represented by individuals < 39 mm TL, while large individuals (adults) were represented by individuals > 40 mm TL. For *C. gilchristi,* small (juvenile) individuals were < 49 mm TL. A canonical correspondence analysis (CCA) was used to relate the patterns of the goby species distribution and abundance with that of the measured environmental variables using RStudio (1.3.1093). Abundance data was first log transformed before analysis. Environmental variables included in the analysis were bottom water temperature (°C), salinity (‰), turbidity (NTU), organic content (%), dissolved oxygen (mg/l), mud (%), sand (%) and prey. For prey (data taken from Chapter 2), only organisms that were dominant in the diet of the different species (Chapter 5) were included, which were amphipods, chironomids, cyclopoids, ostracods, bivalves and mysids.

Results

Environmental characteristics

In summer and autumn temperature decreased from the mouth to the uppermost site (Figure 6.1a). Temperatures were lower in spring and winter and were fairly even throughout the estuary. Salinity decreased along the length of the estuary in all seasons (Figure 6.1b). Turbidity was consistently highest 14 km from the mouth (Figure 6.1c). Dissolved oxygen showed the greatest variability 10 km from the mouth in the middle reaches, with supersaturated conditions recorded in autumn, and anoxic conditions in summer (Figure 6.1d).

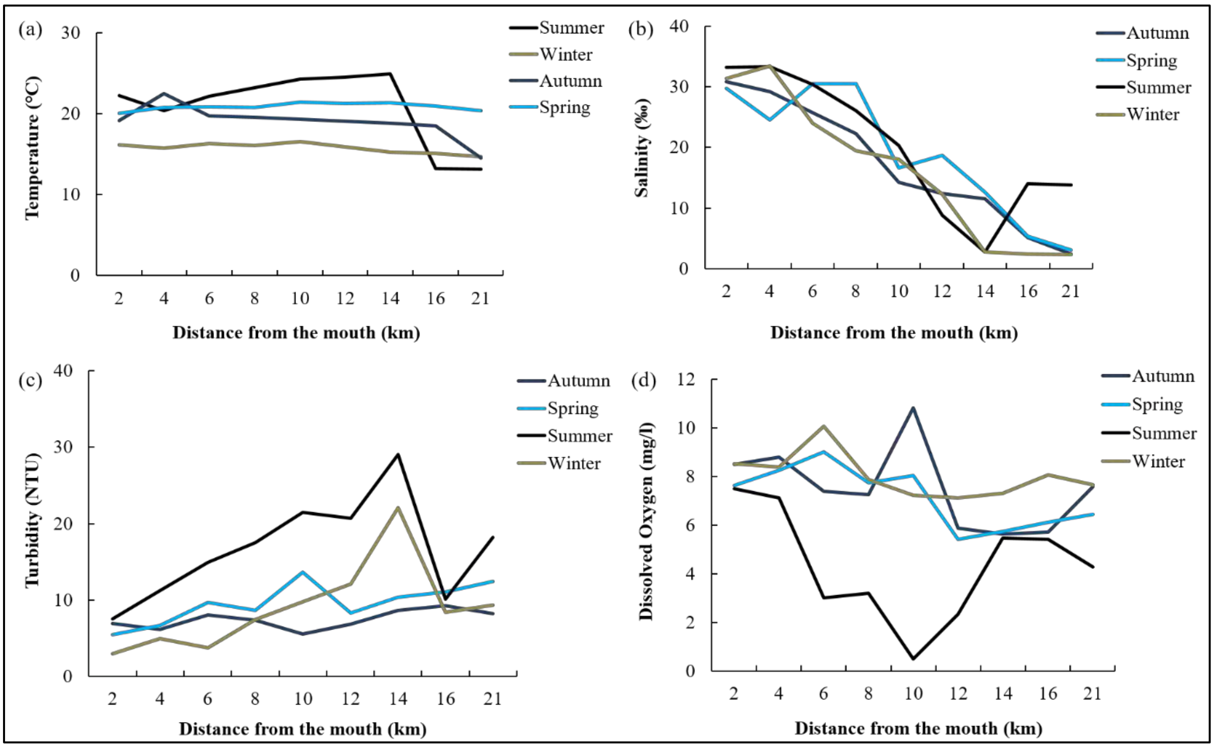


Figure 6.1: Seasonal changes in mean environmental variables along the length of the Sundays Estuary

Grain size composition changed along the length of the estuary (Figure 6.2). The contribution of medium sand increased with distance from the mouth. In contrast, mud and very fine sand were highest at the mouth and lower reaches.

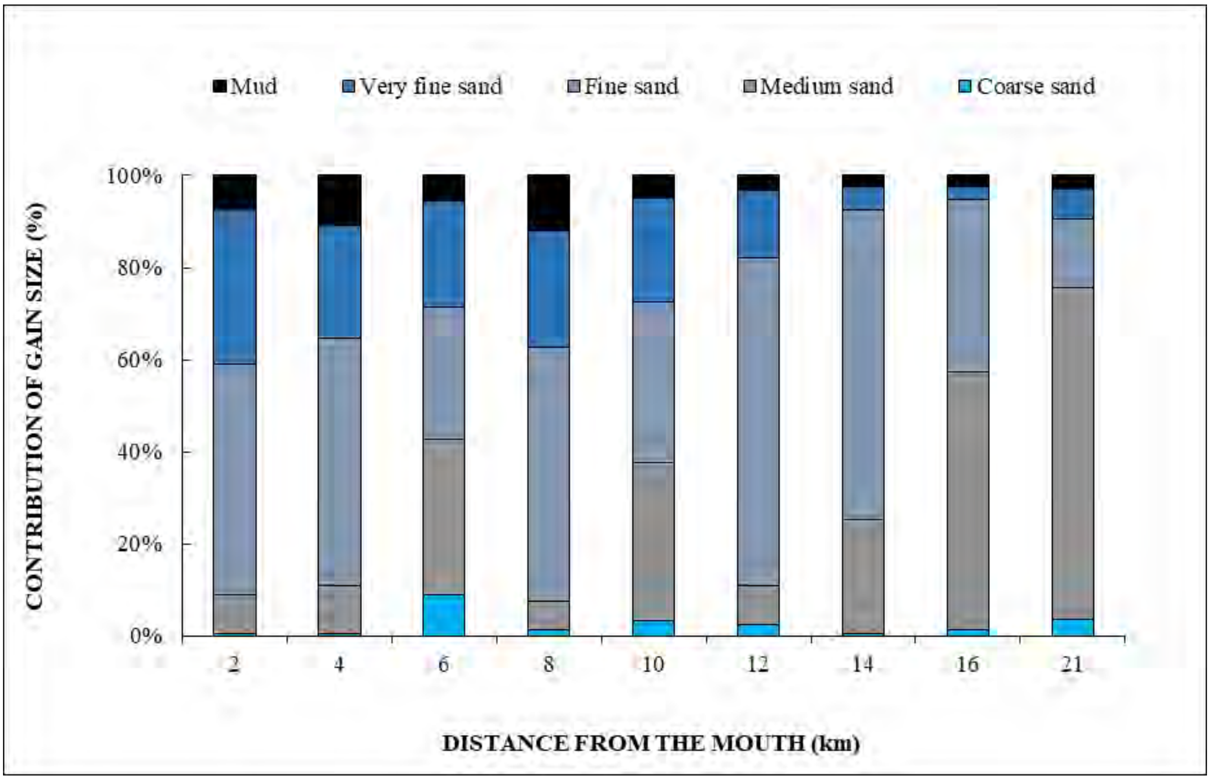


Figure 6.2: Percent contribution of benthic sediment grain size from the different sites of Sundays Estuary

Temporal trends in species composition

The sampling sites were divided into four reaches/regions based on the changes in physicochemical variables (Chapter 2). The first sampling site (2 km from the mouth) represented the mouth, sites 4 and 6 km from the mouth (Sites 2 and 3) represented the lower reach, sites 8–14 km from the mouth (Sites 4–7) represented the middle reach and the upper reach was represented by sites 16 km (Site 8) and 21 km (Site 9) from the mouth.

Psammogobius knysnaensis was recorded in all regions in all seasons (Table: 6.1). The species was highly abundant in the mouth (2 km) except for winter, where only a few individuals were recorded in this site. Though both small and large size individuals were recorded in the mouth region, the region was mostly represented by small size individuals based on the mean size except for winter and spring. In the middle and upper reaches, few individuals were recorded, and they were mostly represented by large size individuals. In all seasons, the mean size increased with distance from the mouth (Table: 6.2).

Cafrogobius gilchristi was highly abundant in the lower reach (4–6 km) and mostly represented by large size, mature individuals. The species was recorded in all regions except for autumn and summer, when it was not recorded in the upper reach (16–21 km). *Glossogobius callidus* had high occurrence in the upper reach, more particularly in the last sampling site. During summer, the species was only recorded in the upper reach, while in other seasons it was recorded in all regions. During summer the species was mostly represented by small size individuals.

Table 6.1: Catch per unit effort (fish/haul) of *Psammogobius knysnaensis*, *Caffrogobius gilchristi* and *Glossogobius callidus* caught per season in the Sundays Estuary. Aut = Autumn, Wi = winter, Sp = Spring and Su = Summer.

		P. knysnaensis				***C. gilchristi***				***G. callidus***			
Distance from the mouth (km)	**Site**	**Aut**	**Wi**	**Spr**	**Su**	**Aut**	**Wi**	**Spr**	**Su**	**Aut**	**Wi**	**Spr**	**Su**
Mouth													
2	1	6	5	19.5	20	2.5	0.5	1	0.5	2.5	1.6	-	-
Lower reaches													
4	2	3.5	4.5	4.5	-	23	5	59	17.5	1	0.5	-	-
6	3	-	6	1.5	2	5.5	31.5	13.5	31.5	2.5	-	0.5	-
Middle reaches													
8	4	-	5.5	1.5	1.5	0.5	-	1.5	1	-	-	-	-
10	5	1.5	0.5	-	-	1	-	-	-	-	-	-	-
12	6	-	-	0.5	-	-	-	-	-	2	0.5	-	-
14	7	-	0.5	-	-	-	0.5	-	-	-	-	0.5	-
Upper reaches													
16	8	-	-	1	-	-	0.5	-	-	1.5	6.5	1	1
21	9	-	1	0.5	-	-	-	4	-	4	3.5	11	57
Total		**11**	**23**	**29**	**23.5**	**32.5**	**38**	**79**	**50.5**	**10**	**11**	**13.5**	**57**

Table 6.2: Mean size (mm TL) of *Psammogobius knysnaensis*, *Caffrogobius gilchristi* and *Glossogobius callidus* caught per season in the Sundays Estuary (March 2018 to September 2019). Adults are shaded in grey.

		P. knysnaensis				***C. gilchristi***				***G. callidus***			
Distance from the mouth (km)	**Site**	**Aut**	**Wi**	**Sp**	**Su**	**Aut**	**Wi**	**Sp**	**Su**	**Aut**	**Wi**	**Sp**	**Su**
Mouth													
2	1	27.5	36.8	38.9	28.3	34.6	25.0	50.0	30.0	25.0	27.5		
Lower reaches													
4	2	30.3	33.4	46.0		31.6	47.2	44.1	50.5	22.3	25.4		
6	3		42.4	44.3	33.3	44.6	35.8	43.3	38.6	32.4		47.0	
Middle reaches													
8	4		51.4	45.7	35.3	34.0		48.0	30.0				
10	5	49.3	47.0			37.5							
12	6			49.0						51.8	41.0		
14	7		54.0				105					27.0	
Upper reaches													
16	8			47.5			25.0			16.7	43.4	27.0	27.0
21	9		54.5	40.0				34.3		38.9	40.9	35.0	28.4

Distribution and abundance

Species distribution changed along the length of the estuary (Figure 6.4). Although both *P. knysnaensis* and *C. gilchristi* were primarily distributed in the lower reaches and mouth region, they partitioned habitat within this area, with *P. knysnaensis* abundant in the mouth region (Site 1, 2 km from the mouth), with a mean abundance of 24.20 individuals/trawl. *Caffrogobius gilchristi* were abundant at Site 2 (30.50 individuals/trawl) and Site 3 (26.00 individuals/trawl) in the lower reaches. *Glossogobius callidus* was abundant in the upper reaches, with the greatest abundance recorded in the uppermost site (33.10 individuals/trawl). All three species were recorded in very low numbers in the middle reaches (Table 6.1, Figure 6.4).

In the mouth (Site 1), *P. knysnaensis* was represented mostly by small (< 39 mm) juveniles, with large (> 40 mm) mature individuals dominant in the lower reaches (Site 2) (Figure 6.4). In the middle and upper reaches, the species was mostly represented by large sized mature individuals. *Caffrogobius gilchristi* was represented by both small (< 49 mm) juveniles and large (> 50 mm) adults in all three reaches (lower, middle and upper), with very few individuals recorded in the mouth. In the lower reaches, *G. callidus* was mostly represented by small sized (< 39 mm) juveniles, and the species was not recorded in the mouth. Although the species was represented by both small (< 39 mm) and large (> 40 mm) individuals in the middle and upper reaches (Figure 6.4), in spring juveniles dominated in the upper reaches (Table 6.2).

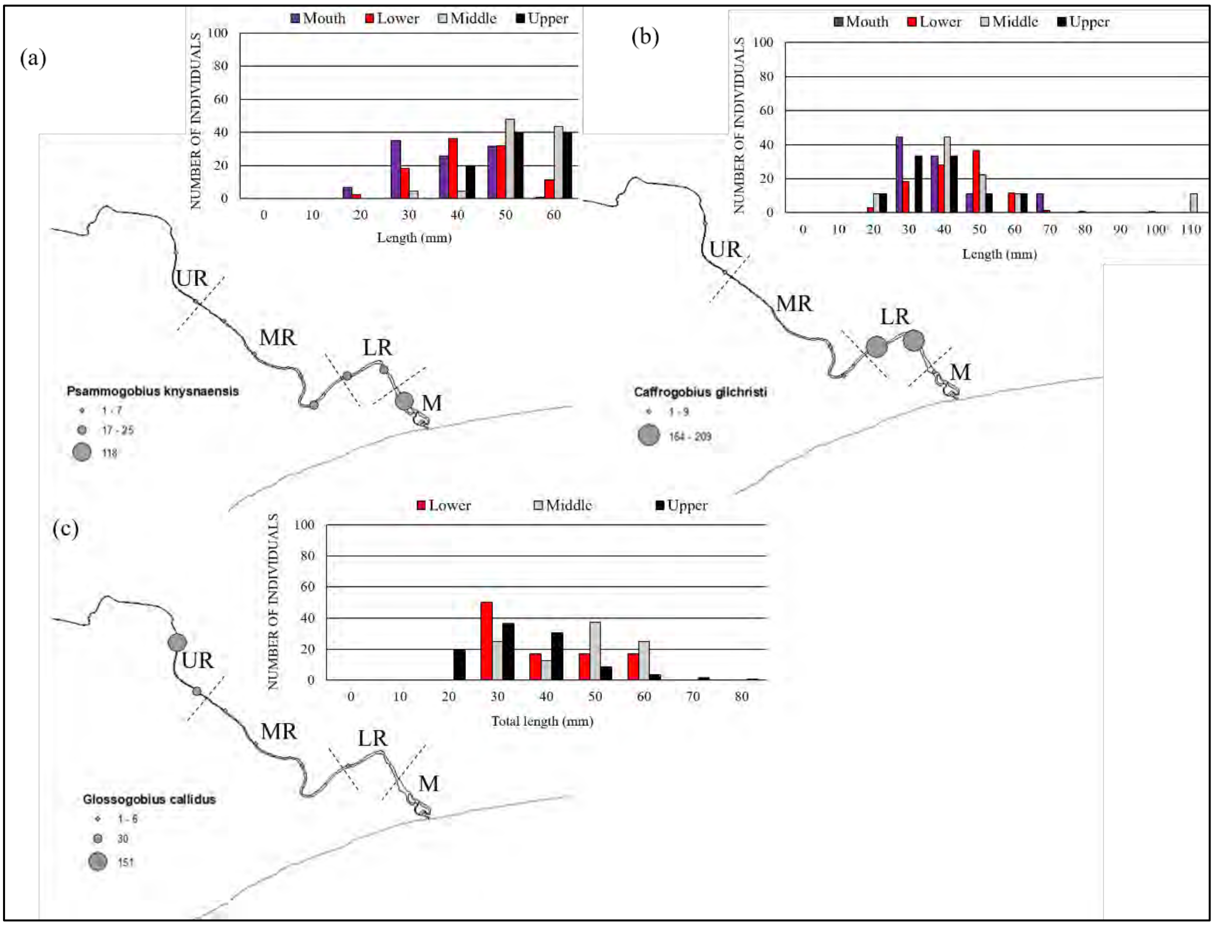


Figure 6.4: Distribution, abundance and length frequency of *Psammogobius knysnaensis* (a), *Caffrogobius gilchristi* (b) and *Glossogobius callidus* (c) in the Sundays Estuary (March 2018 to September 2019). M = mouth, LR = lower reach, MR = middle reach and UR = upper reach. Blue star in the x axis represents length at 50% maturity

The first, second and third components of CCA explained 57%, 18% and 0.5% of varience respectively (Figure 6.5). The dominance of small *P. knynsaensis* at Site 1 in the mouth was positively correlated with the presence of bivalves (Figure 6.5). High salinity, dissolved oxygen and the presence of mysids also influenced the distribution of *P. knysnaensis* (particularly large *P. knysnaensis*). *Caffrogobius gilchristi* (small and large individuals) were associated with the lower reaches (Sites 2 and 3), and with high temperatures and higher mud (mud and fine sand together) content. Small and large sized *G. callidus* were abundant in the upper reaches at Sites 8 and 9, and were associated with high turbidity and amphipods (Figure 6.5).

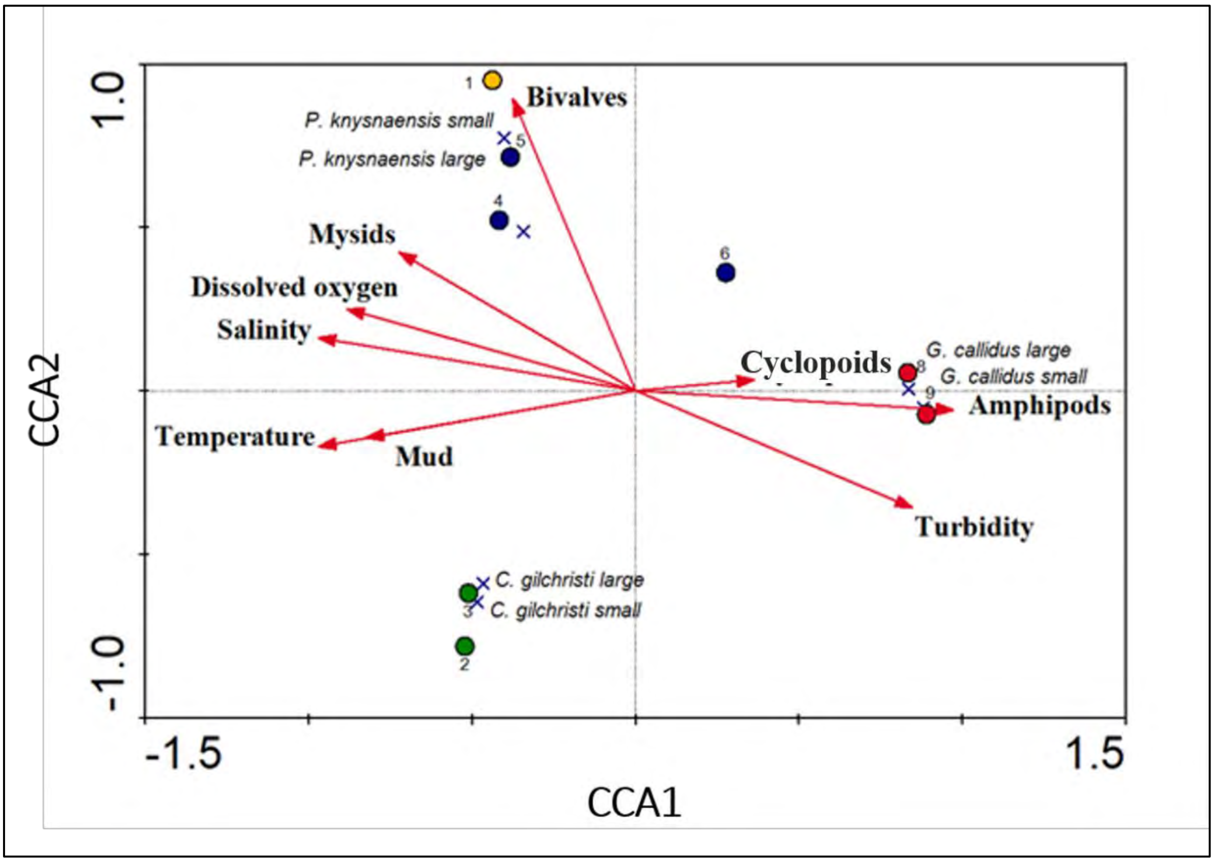


Figure 6.5: Canonical correspondence analysis (CCA) showing the influence of environmental variables on the species distribution. 1 = small size individuals, 2 = large size individuals

Discussion

This study aimed to determine the drivers of the distribution and abundance of three co-existing goby species along a longitudinal gradient in the Sundays Estuary. Prey availability, salinity, turbidity, temperature and sediment characteristics along the estuarine gradient were related to species-specific habitat associations.

In all three species, there were low abundances recorded in the cold winter months. High species abundance recorded in other months could be the result of reproductive activity taking place during the warmer months (Chapter 3). Small size individuals were mostly recorded during the summer period, decreasing in autumn. The same behaviour was observed in a study by Wasserman (2012), who observed high larval fish abundance in summer. Both juvenile and adult *Glossogobius callidus* were mostly recorded in the low salinity upper reaches, with high turbidity and amphipods influencing their distribution. Amphipods are one of the preferred prey items in the diet of this species (Chapter 5; Wasserman, 2012; Mofu et al., 2019). This distribution pattern was also evident in the St Lucia Estuary, where *G. callidus* was mostly recorded in the upper reaches, which had low salinity and high turbidity (Harris and Cyrus, 2000). Similarly, in the Kariega Estuary, *G. callidus* was mostly recorded in the upper reaches, with a high dominance of juvenile fishes during summer months (Ter Morshuizen and Whitfield, 1994). *Glossogobius callidus* is classified as a freshwater and estuarine species and this could explain why the species preferred the low salinity in the upper reaches of the estuaries (Whitfield, 2019). However, as the species can tolerate high saline environments (Nodo et al., 2018), the preference for the upper reaches is likely due to prey availability, high turbidity and for use as nesting sites, as high abundances of larval fish have been recorded in these regions (Wasserman, 2012). Turbidity is mostly used by fish as a refuge from predators (Carter et al., 2009).

Psammogobius knysnaensis and *G. callidus* had similar diets in the upper reaches in both the juvenile and adult stages, with cyclopoids, amphipods and chironomids in common. Interestingly, *P. knysnaensis* caught in the mouth and lower reaches had a different diet to those caught in the upper reaches, and preyed on ostracods and bivalves, with bivalves comprising an important component in the diet of large size individuals of *P. knysnaensis* (Chapter 5). This means that *G. callidus* potentially outcompetes *P. knysnaensis* in the upper reaches. The abundance of *P. knysnaensis* in the sandy mouth region suggests that habitat partitioning is the main strategy by which they avoid competition with *G. callidus*. *Psammogobius knysnaesis* has been known to occur as a symbiont in sand prawn *Callianassa krausii* burrows (Moyo, 2014), which also may explain their preference for the sandy mouth region. A similar trend was observed with *Lithognathus lithognathus*, where the species was found to be highly abundant in association with its prey *Callianassa krausii* in the sandy lower reaches of estuaries (Bennett et al., 2015). Burrows provide protection from predators and food (Moyo, 2014; Kohda et al., 2016). The distribution pattern of these two species persists in temperate South African estuaries. Very few *G. callidus* were recorded in the lower reaches, compared to those in the upper reaches of the Kariega Estuary (Whitfield and Paterson, 2003; Richardson et al., 2006; Vorwerk et al., 2008; Nodo, 2018). In both the Swartkops and Kariega estuaries, *P. knysnaensis* have been mostly recorded in the sandy lower reaches near the mouth (Melville-Smith and Baird, 1980; Ter Morshuizen and Whitfield, 1994; Vorwerk et al. 2008; Nodo et al., 2018).

Examples of habitat partitioning when there is dietary overlap show that it is a common strategy in functionally similar gobies. For example, in the Mondego Estuary in Portugal, polychaetes are common in the diet of both the common goby *Pomatoschistus microps* and sand goby *Pomatoschistus minutus*, and in order to reduce competition they exploit the same resources in different habitats (Leitão et al., 2006). Schofield (2003) showed that the code goby *Gobiosoma robustum* (Ginsburg, 1933) and clown goby *Microgobius gulosus* (Girard, 1858) occupied different habitats to avoid competition over available resources. The former species preferred seagrass, while the latter species preferred bare mud (Schofield, 2003). This mechanism, however, is not ubiquitous amongst all gobies, as in Lake Hamana (Japan), the dominance of two *Acentrogobius* species in different habitats was found to be a result of habitat preference rather than competition. *Acentrogobius* spp 1 prefers all sediment types whereas *Acentrogobius* spp 2 avoided areas with fine sand (Horinouchi, 2008).

Although there was greater overlap in the distribution of *P. knysnaensis* and *C. gilchristi*, habitat partitioning between *P. knysnaensis* and *C. gilchristi* (in terms of the two species dominating in different sites) also enables the juveniles of these two species to feed on similar prey (cyclopoids and mysids). *Caffrogobius gilchristi* was abundant in the lower reaches, particularly in a site with a higher mud (clay) content. In the Kariega Estuary, *C. gilchristi* was also associated with muddy habitats in the lower and middle reaches (Richardson et al., 2006; Bailey and James, 2013; Nodo et al., 2018).

All three species were recorded in very low numbers in the middle reaches compared to the other two reaches, even though *C. gilchristi* is known to be abundant in the middle reaches of other estuaries (Nodo et al., 2018). The middle reaches of this estuary are eutrophic, with low dissolved oxygen (Lemley et al., 2018) potentially making them a poor habitat for gobies. Low dissolved oxygen has the potential to reduce prey availability and growth rate (McNatt and Rice, 2004; Powers et al., 2005; Fox et al., 2009; Campbell and Rice, 2014). Low dissolved oxygen can also result in mortality of fish eggs and suppression of gonad development and this can be exacerbated in benthic fishes (Breitburg et al., 2003; Thomas et al., 2007). All of these adverse effects may explain why the three species seemed to avoid this region of the estuary.

Intraspecific habitat partitioning by size was only apparent in *P. knysnaensis*, with juveniles dominating the mouth region (Site 1) and adults the lower reaches (Site 2). The diet of all three species changed with ontogeny (Chapter 5) and this may be why the juveniles and adults were abundant in the same areas. Ontogenetic changes in diet may limit intra-specific competition for resources, and this may explain why habitat preference did not change with ontogeny for *G. callidus* and *C. gilchristi*. When a species' diet does not change with ontogeny, they tend to occupy different habitats at different sizes to avoid intraspecific competition (Clark and Pessanha, 2015).

Conclusion

Patterns in the distribution and abundance of the three goby species in the Sundays Estuary were driven by both biotic variables (prey availability, competition) as well as abiotic characteristics (salinity, turbidity, temperature and sediment characteristics). As hypothesised, *P. knysnaensis* and *C. gilchristi* occupied different habitats. *Psammogobius knysnaensis* and *C. gilchristi* were associated with the mouth region and lower reaches, with prey availability (bivalves and mysids), sediment type and low turbidity being the driving factors for the former species in the mouth region. Mud and temperature were the driving factors of the latter species' abundance in the lower reaches. *Glossogobius callidus,* although overlapping in diet with *P. knysnaensis*, was abundant in the uppermost sites, which were characterised by high turbidity, low salinity and a high abundance of amphipods.

CHAPTER SEVEN

General discussion

Estuaries are fluctuating aquatic environments and so few species are able to complete their entire life-cycle in estuaries (Whitfield and Harrison, 2020). Of the 172 fish species that occur in southern African estuaries, only 43 species are able to breed in the estuaries (Whitfield, 2019). Of the 43 species, 25 belong to the Gobiidae family (Whitfield, 2019). Gobies occur in high numbers and are a dominant component of the demersal fish assemblage in South African estuaries (Richardson et al., 2006; Bailey and James, 2013; Nodo et al., 2018). Although gobies are an important component of the fish community in estuaries, very few studies have focussed on the biology and mechanisms enabling the co-existence of gobies in South African estuaries. This study has demonstrated that their high abundance and high diversity within estuaries is promoted through resource partitioning.

The findings of this study suggests that habitat partitioning between the three goby species, most abundant in warm-temperate South African estuaries, is the major driver promoting their coexistence in these estuaries. This was mostly influenced by prey availability, sediment characteristics, salinity, temperature and turbidity. The estuarine and marine resident, *Psammogobius knysnaensis,* although distributed throughout the estuary (Figure 7.1) was most abundant in the mouth region, with bivalves and mysids (prey availability) and sandy sediment being the key factors in their high abundance in this region. The consumption of larger high energy gain prey increased with fish size in this species, with the consumption of bivalves, mysids, crabs, amphipods and chironomids increasing with size. Bivalves and ostracods, which were abundant in the sandy mouth region, were an important component in the diet of this species. *Psammogobius knysnaensis* also lives in the burrows of the sand prawn *Callianassa krausii,* which provide protection from predators (Moyo, 2014), which further explains their preference for the sandy mouth region.

The estuarine migrant *Caffrogobius gilchristi*, although also distributed throughout the estuary, was highly abundant in the muddy lower reaches. Among the small sizes the species specialised in feeding on cyclopoids but among the adult stages it fed on mysids, amphipods, crabs and gastropods. Their consumption of crabs and gastropods is promoted by large lower jaw lengths, which enables their jaws to have a high crushing force (Sibbing and Nagelkerke, 2001).

Gastropods and crabs, which were abundant in the diet of this species, were also abundant in the environment where the species was collected. The species occurs in sympatry with *Caffrogobius nudiceps* (Nodo, 2022), with amphipods, copepods, isopods and brachyura common in the diet of both species (Bennett et al., 1983; Whitfield, 1989). However, *C. gilchristi* outcompetes *C. nudiceps*, as very few individuals of the latter have been captured in this region (Nodo, 2022). Before this study, very little observation had been recorded on the biology and ecology of this species, despite its high abundance in South African temperate estuaries.

The estuarine and freshwater species *Glossogobius callidus* was also recorded throughout the estuary but was most abundant in the uppermost sites, with prey availability (amphipods and cyclopoids) and high turbidity being the driving factors of their high preference for this region. These two prey items were an important component of the diet of this species in the upper reaches. In the lower reaches, they were only found as small individuals and this could be explained by the fact that amphipods and chironomids, which are the preferred prey for the adult fish, were not recorded in this region. Morphological characteristics suggest that the species is a generalist feeder, and this was proven to be true in this study, with this species feeding primarily on cyclopoids, amphipods and chironomids. The biology (diet) of this species has been studied in a freshwater environment (Mofu, 2016) but in estuaries only the diet of the larval and early juvenile stages have been studied (Wasserman, 2012).

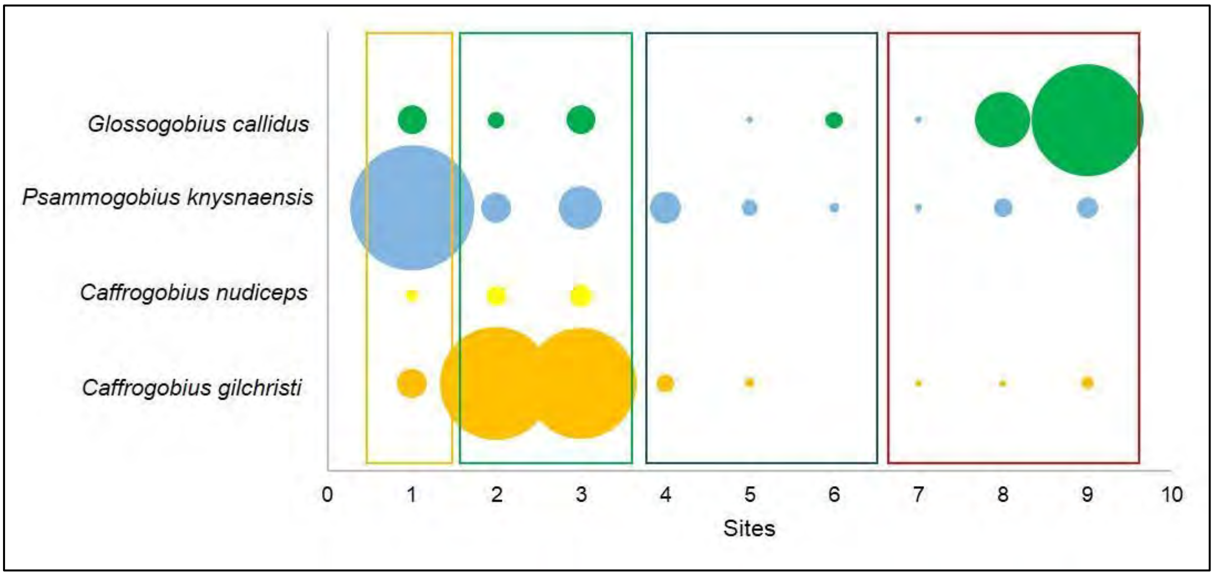
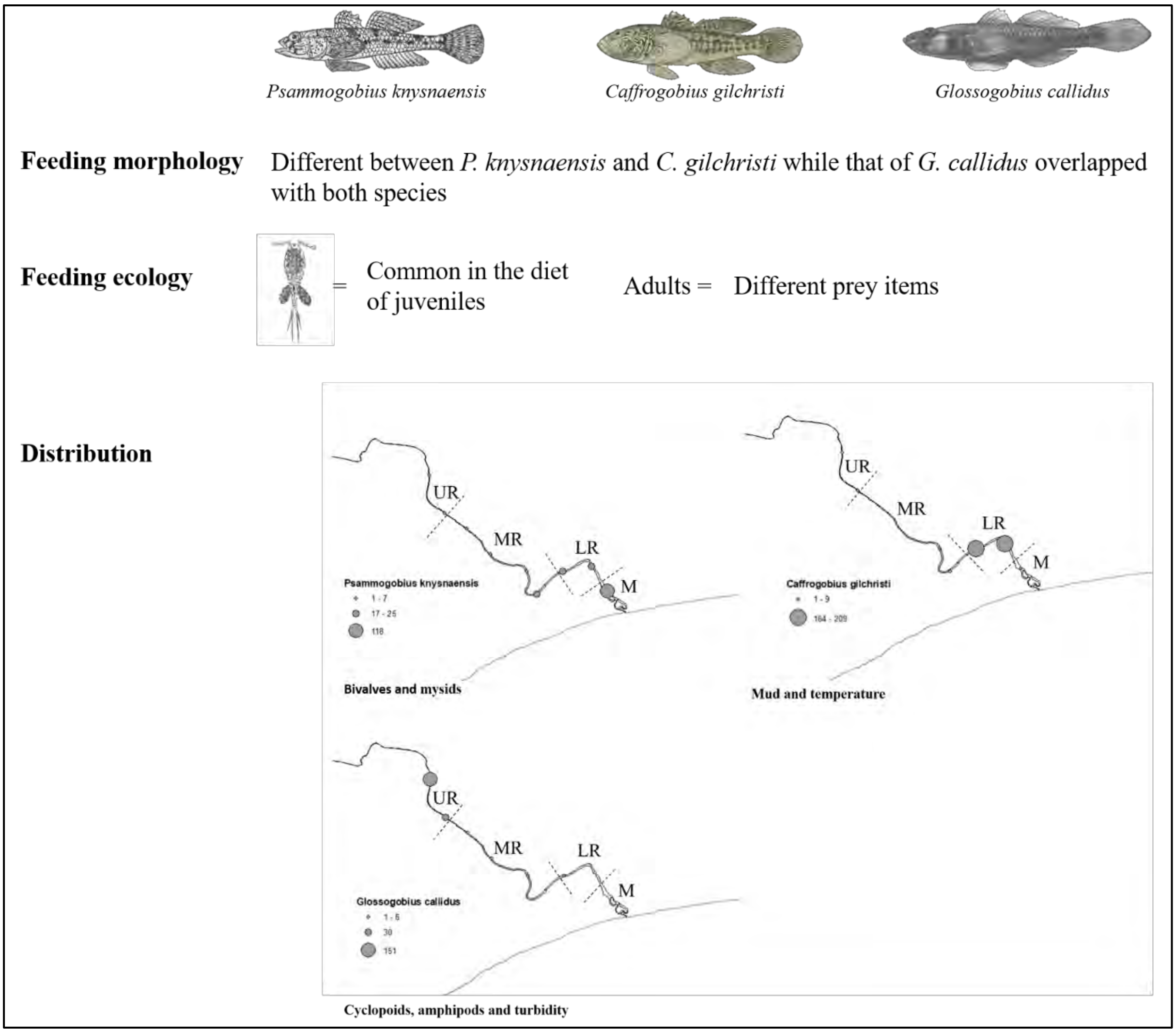


Figure 7.1. The distribution and abundance of gobies recorded in the Sundays Estuary (data for *C. nudiceps* from Nodo, 2022). Yellow line = mouth, green line = lower reaches, blue line = middle reaches, maroon line = upper reaches. Bubbles show individuals per 1000m^2

Habitat squeeze through eutrophication and permanently low dissolved oxygen in the middle reaches of the estuary, primarily as a result of nutrient enrichment from citrus farming, resulted in low abundances of all three species in the middle reaches. Since *G. callidus* is known to be abundant in the upper and middle reaches of other estuaries (Bailey and James, 2013; Richardson et al., 2016), low dissolved oxygen could have contributed to the relatively few large sized individuals in the middle reaches. Low dissolved oxygen could have also contributed to the absence of small sized *P. knysnaensis* in the middle and upper reaches. Low prey availability in the middle reaches also could have contributed to low fish abundance in this region.

The dominance of different species in different habitats enables them to reproduce at the same time, as they do not compete for nesting space. Their ability to feed at different sites has reduced food competition for energy investment needed for reproduction. Because both *P. knysnaensis* and *C. gilchristi* start to reproduce at the same size they have the potential to compete for nesting sites, as they all use the underside of rocks and other hard surfaces to lay their eggs (Wasserman et al., 2015; Wasserman et al., 2017; Whitfield, 2019). A summary of how these species manage to coexist is provided in Figure 7.2. A life history summary of the three study species is provided in Table 7.1.



Feeding morphology Different between *P. knysnaensis* and *C. gilchristi* while that of *G. callidus* overlapped with both species

Feeding ecology = Common in the diet of juveniles Adults = Different prey items

Distribution

Figure 7.2: Summary of the coexistence between *Psammogobius knysnaensis, Caffrogobius gilchristi* and *Glossogobius callidus* in the Sundays Estuary. M = mouth, LR = lower reach, MR = middle reach and UR = upper reach. Numbers next to the bubles represent fish abundance.

Table 7.1: Life history of *Caffrogobius gilchristi, Glossogobius callidus* and *Psammogobius knysnaensis*

	Caffrogobius gilchristi	***Glossogobius callidus***	***Psammogobius knysnaensis***
Category (Whitfield, 2019)	Ib (breeds mainly in estuaries also marine)	I (breeds mainly in estuaries also freshwater)	I (breeds mainly in estuaries also marine)
Distribution (Whitfield, 2019)	Endemic to Southern Africa (Olifants to Durban)	Endemic to Southern Africa (Mozambique to the Swartvlei region of the Western Cape)	Endemic to Southern Africa (Port Nolloth to KwaZuluNatal)
Spawning season	Spring and summer (this study)	Spring and summer (Mofu, 2016)	Spring and summer (this study)
LM50	45 mm TL both male and female (this study)	Male 72 mm TL Female 70 mm TL (Mofu, 2016)	Male 45 mm TL Female 40 mm TL (this study)
Morphology (this study)	High caudal peduncle depth, body depth and lower jaw length. Specialist feeder	Small gape size, velocity suction, hyoid length and relative gape area. Generalist feeder	High gut length, gape area, hyoid length, relative gape area, and high velocity suction. generalist feeders
Diet juveniles (this study)	Cyclopoids dominant (20–39 mm)	Cyclopoids and amphipods (20–39 mm)	Cyclopoids and ostracods (20–39 mm)
Diet adults (this study)	Cyclopods, amphipods, mysids, brachyura, gastropods	Amphipods and chironomids (40–59 mm)	Bivalves and cyclopoids (> 39 mm)
Dominant habitat juveniles (this study)	Lower reaches (high temperatures, salinity, mud, gastropods and mysids)	Upper reach (turbidity, cyclopoids, amphipods, polychaeta, brachiopoda and decapods	Mouth (salinity, dissolved oxygen, mysids, bivalves, gastropods, polychaetes and calanoids)
Dominant habitat adults (this study)	Lower reaches (high temperatures, salinity, mud, calanoids, mysids, polychaetes, gastropods, decapods)	Upper reaches (turbidity, cyclopoids, amphipods, polychaeta, brachiopoda and decapods	Mouth (salinity, dissolved oxygen, mysids, bivalves, gastropods, polychaetes and calanoids)
Seasonal partitioning (this study)	None	None	None

In conclusion, the three study species partitioned their resources by occupying different habitats. Prey availability, together with sediment characteristics and temperature, promoted habitat partitioning between these species. This reduced competition by enabling them to use resources from different habitats. Cyclopoids were common in the diet of all species, however, different morphological structures enabled the different species to feed on different prey items. The species were shown to reproduce at the same time and this could have been promoted by their abundance in different sites. Anthropogenic impacts, such as eutrophication and low dissolved oxygen, resulted in low species abundance in the middle reaches and thereby reduced the habitat available for gobies in the Sundays Estuary. Future studies should focus on the species' prey availability and diet across seasons, as well as the reproduction of *G. callidus* in estuaries, and the nesting sites and reproductive behaviour of *C. gilchristi.* Predator avoidance, which was not taken into consideration in the current study, could have also contributed to shaping the distribution of these species. Using gut content analysis together with the stable isotope analysis could have provided more comprehensive information on food partitioning between the study species.

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