Spatial habitat partitioning enables coexistence of three species of Gobiidae in South African estuaries

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INTRODUCTION

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) and are one of only two fish families (the other being Mugilidae) that are well represented in cool-temperate, warm-temperate and subtropical estuaries (Harrison 2003; Figure S1). Estuaries are fluctuating aquatic environments and as a result few species are able to complete their entire life-cycle in estuaries (Whitfield and Harrison, 2020). Of the 172 fish species that occur in South Africa's 290 estuaries, only 43 species are able to breed in estuaries, with 25 from the Gobiidae family (Whitfield, 2019). 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.

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 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). For some species, resource competition is avoided by closely related species feeding on similar resources but occupying different habitats (Tilney and Hecht, 1990; Islam et al., 2006).

Specialisation in resource use minimises competition, thereby enabling stable coexistence between similar competing species in aquatic systems (Guo et al., 2014), and explains how multiple species are able to coexist in aquatic systems in the same habitats. Niche differentiation plays an important role in maintaining species diversity at different scales (Leibold and McPeek, 2006). Niche differentiation can be promoted by diversification of morphological structures responsible for feeding (Burres 2016; Porreca et al. 2017). An organism's morphology influences its capacity to locate, chase, capture and process a prey. As such, functional morphological traits have been used to predict resource partitioning in a wide variety of fishes (e.g. Wainwright and Richard, 1995; Sibbing and Nagelkerke, 2001; Burress, 2016; Mittelheiser et al., 2022). For example, in a large-scale study of 367 species from 20 orders from the Nearctic, Neotropical and Afrotropical regions, Keppeler et al. (2020) showed that gut length, tooth shape, mouth width, mouth orientation and body depth are strong predictors of trophic level.

The Gobiidae family provides excellent subjects to study trait-based mechanisms of resource partitioning among the estuarine resident and zoobenthivorous functional groups in estuaries to explain their coexistence and abundance in South African estuaries. This study aims to describe resource partitioning and co-existence of three goby species which are the most abundant gobies found in 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). Interspecific differences in feeding morphology, feeding ecology, and habitat partitioning were investigated to determine what facilitates the co-existence and success of these three species in South African estuaries.

MATERIALS AND METHODS

Study area

Sampling was conducted between February 2018 and September 2019 in the Sundays Estuary, which is a predominantly open estuary flowing into Algoa Bay in the Indian Ocean at 33°43'14.5"S 25°51'10.4"E, 35 km north-east of Gqeberha, South Africa (Figure 1). The Sundays Estuary is approximately 21 km long and because of agricultural activities in the catchment is permanently eutrophic (Lemley et al. 2017). Dissolved oxygen concentrations are consistently low in the middle reaches (average 5 mg/l), with hypoxic conditions (>1 mg/l) recorded in summer (Nodo et al. 2023a). Salinity decreases from an average of 30 in the mouth to 1 in the upper reaches (Nodo et al. 2023a).

Study species

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). Prey items present in its diet include amphipods, polychaetes, insect larvae, isopods, copepods, cumacea, decapods and ostracods (Bennett, 1989, Whitfield, 1988). *Caffrogobius gilchristi* is also 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 and lower reaches of estuaries (McGregor et al. 2018; Nodo et al. 2018). 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).

Glossogobius callidus 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). In estuaries, *G. callidus* are mostly recorded in the saline upper and middle reaches (Ter Morshuizen and Whitfield 1994). Amphipods, cyclopoids and chironomid larvae dominate the diet of this species in the early life-stages (Vumazonke, 2008; Wasserman, 2012). All three species reproduce in spring and summer and reach 50% maturity at approximately 40 mm TL (Boullé 1989; Ndaleni et al. 2024).

Habitat partitioning

To determine spatial and temporal trends in the abundance of the three species in the estuary fish were sampled using a 3 m shoeless beam-trawl with a 14 mm mesh size beam trawl during daylight hours from July 2017 to September 2019 for a total of twelve sampling occasions. Fish were sampled as part of a larger study on the demersal fish community of the Sundays and Swartkops estuaries and adjacent nearshore (see Nodo et al. 2023a,b; Nodo et al. 2024 for sampling details). The first sampling site; Site 1 (2 km from the mouth) represented the mouth, sites 4 and 6 km from the mouth (Sites 2 and 3) represented the lower reaches, sites 8–14 km from the mouth (Sites 4–7) represented the middle reaches and the upper reaches were represented by sites 16 km (Site 8) and 21 km (Site 9) from the mouth (Figure 1). At each sampling site the net was towed 20 m behind the boat at a constant towing speed of approximately 2 knots for three minutes. One trawl haul was done at each site, covering a distance of approximately 200 m. Fish were identified and measured to the nearest millimetre total length (TL) and then released alive.

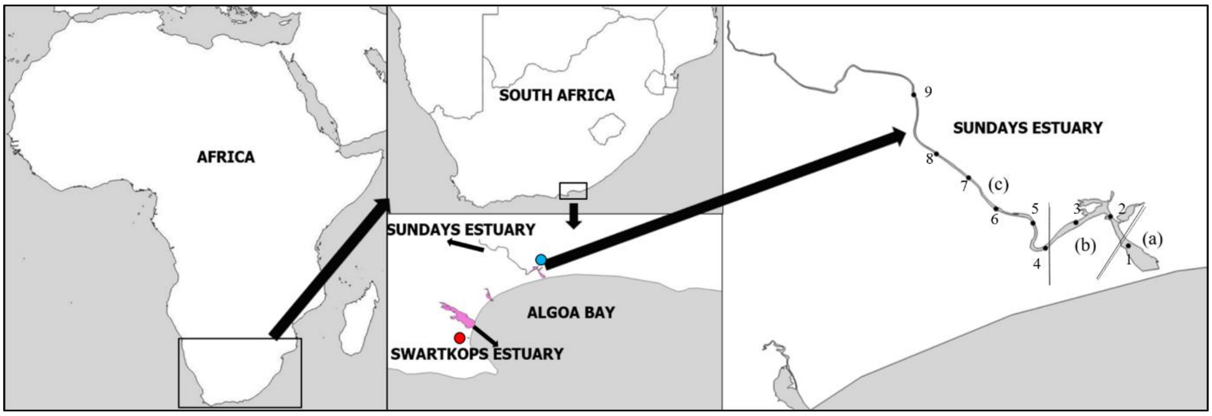


Figure 1: Sampling sites within the Sundays Estuary, Eastern Cape, South Africa. Red dot = Gqeberha, blue dot = Colchester, (a) = Euhaline, (b) = polyhaline and (c) = mesohaline region

At each site bottom water physicochemical parameters (temperature, pH, salinity, depth, turbidity and dissolved oxygen) were recorded using a YSI (6290) multi-parameter probe. Sediment samples were also collected using a cone dredge for particle size and sediment organic content analysis (Nodo et al. 2023a).

Catch-per-unit-effort (CPUE) was calculated as the index of density (fish per 1000 m^2)

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

Feeding morphology

For feeding morphology analysis, a total of 54, 60 and 48 individuals were collected for *P. knysnaensis, C. gilchristi* and *G. callidus* respectively in July 2018 using a small mesh seine net (30 m x 2 m) with a 5 mm bar mesh. Sampling was conducted throughout the estuary until a minimum of 40 individuals of each species were caught. 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 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 (Supplementary material Table S1 and Figure S1). Measurements followed those detailed in Nagelkerke et al. (2018) and Sibbing and Nagelkerke (2001) for cyprinids. Functional morphological traits measured include those responsible for prey location, chasing, capturing, mastication and digestion.

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 (data points that are that significantly differs from other observations). Correlations of variables were calculated for each species to avoid collinearity. Choices were made about which variables to include and exclude. A Principal component analysis (PCA) was then performed on the standardised data to visualise the overall trophic morphology of each fish specimen. 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 using the procedures outlined by Nagelkerke et a. (2018). To compare the overall capacity to feed on different aquatic food types a PCA was performed on the TPs. For each species, mean TPs were calculated and clustered using the pvclust package (Suzuki and Shimodaira 2006), 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. Data analysis was conducted in R statistical software (3.5.1).

Diet

For foregut content analysis fish were collected twice, in spring (September/October 2018) and in winter (August 2019). Fish samples were collected by beam trawling at nine sites in the channel of the estuary (as for habitat partitioning) and supplemented by seine netting (30 m x 2 m with a 5 mm bar mesh) in the littoral zone (as for feeding morphology). This sampling was conducted independently from the habitat partitioning sampling. A total number of 106 *P. knysnaensis*, 103 *C. gilchristi* and 67 *G. callidus* were collected in September/October 2018 and 170 *P. knysnaensis*, 49 C. *gilchrist*i and 81 *G. callidus* in August 2019.

In the laboratory, each fish was measured for total length (TL) to the nearest mm and the foregut of the fish then removed via dissection. 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 following the procedures of Wasserman et al., (2011). For digested prey, head counts were conducted. Relative importance of each invertebrate taxon was assessed using the index of relative importance ($IRI = (\%N + \%V) \times \% F$). 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). Levins niche breadth was compared using %IRI values.

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

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:

$$B_a = (B - 1)(n - 1)$$

Where Ba = standardised niche breadth and n = the summation number of all the prey items.

Prey resources

Mesozooplankton

Mesozooplankton communities were sampled once-off (one replicate) during the day (28 August 2019) using an epibenthic sled at the nine beam trawl 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).

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:

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

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

RESULTS

Habitat partitioning

In total, 541 *C. gilchristi*, 367 *G. callidus* and 421 P. *knysnaensis* were caught. The three species partitioned habitat in spring, summer and autumn, with juvenile and adult *P. knysnaensis* mainly found at Site 1 in the mouth, juvenile and adult *C. gilchriti* at sites 2 and 3 in the lower reaches and juvenile and adult *G. callidus* at sites 8 and 9 in the upper reaches. In winter, *P. knysnaensis* and *G. callidus* were distributed throughout the estuary with only *C. gilchristi* abundant at sites 2 and 3 (Figure 2).

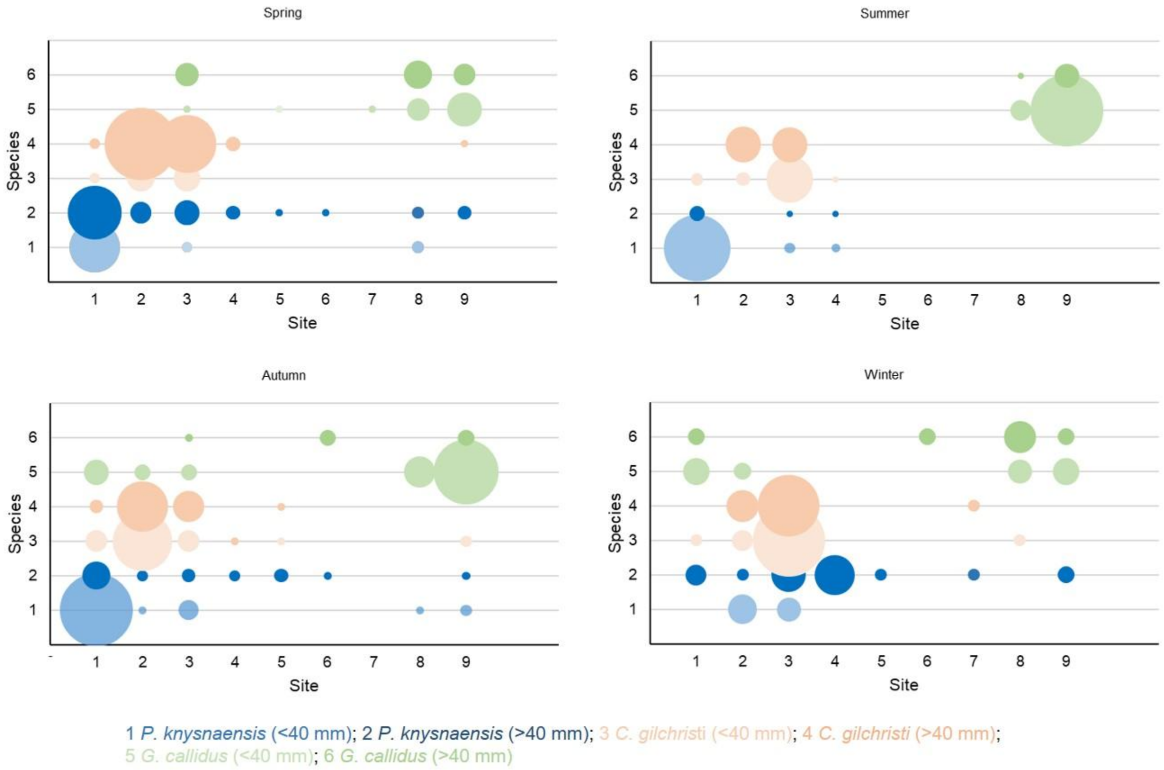
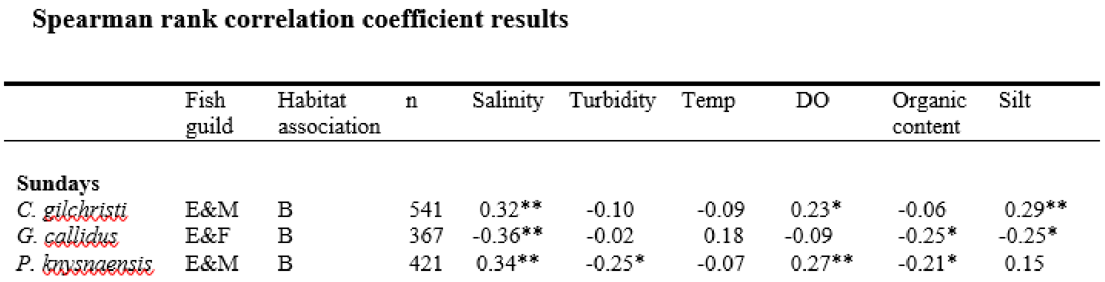


Figure 2. Bubble plots showing the ind. haul $^{-1}$ (CPUE) of juvenile (<40 mm TL) and adult (>40 mm TL) *P. knysnaensis*, *C. gilchristi* and *G. callidus* in the Sundays Estuary

Phakama to add spearman correlations with environmental variables



Spearman rank correlation coefficient results

	Fish guild	Habitat association	n	Salinity	Turbidity	Temp	DO	Organic content	Silt
Sundays									
C. gilchristi	E&M	B	541	0.32**	-0.10	-0.09	0.23*	-0.06	0.29**
G. callidus	E&F	B	367	-0.36**	-0.02	0.18	-0.09	-0.25*	-0.25*
P. knysnaensis	E&M	B	421	0.34**	-0.25*	-0.07	0.27**	-0.21*	0.15

Feeding morphology

The minimum and maximum lengths of *C. gilchristi*, *G. callidus* and *P. knysnaensis* were 17.0 and 84.3 mm, 13.6 and 51.3 mm and 23.5 and 47.9 mm, respectively. The first three dimensions of the PCA ordination of the functional feeding traits of the three species represented 17.0%, 13.8%, and 9.4% of the total variation (Figure 3). The feeding morphology of *P. knysnaensis* separates from the other species, mostly through oral gap diameter, relative gap area, gill arch resistance and gut length. *Glossogobius callidus* and *C. gilchristi* are mostly overlapping and are characterised by pharyngeal jaw symphysis, body roundness, body depth and caudal penducle depth (Figure 3a, c). Some individuals of *G. callidus* separate out and are characterised by opercular volume capacity.

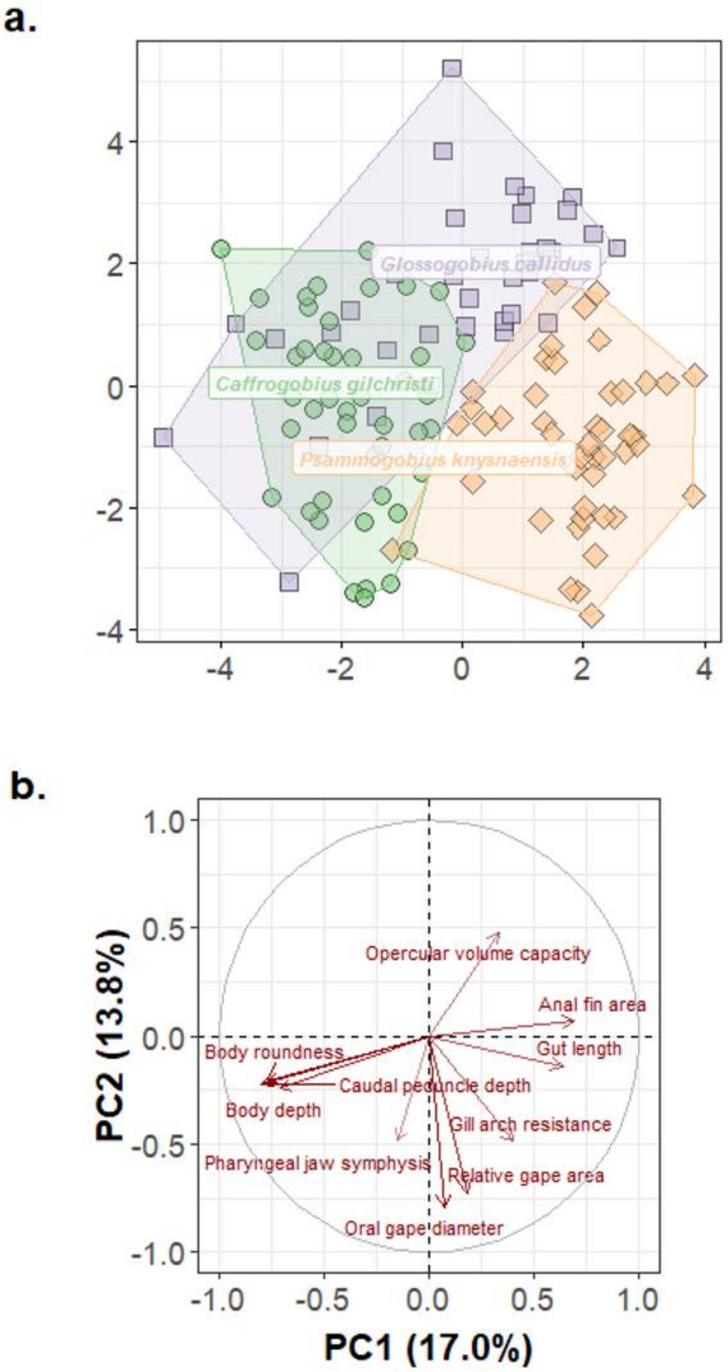


Figure 3: Principal component analysis of the 32 measured functional feeding traits of *Psammogobius knysnaensis*, *Caffrogobius gilchristi* and *Glossogobius callidus* collected from the Sunday Estuary, Eastern Cape, South Africa. A = each marker represents individual fishes and the different colours represent different species. B = direction and size of the loadings of the feeding traits on the ordination

The first two together with the third components of the PCA ordination of the trophic profiles represents 37.2%, 28.3% and 14.6% of the total variation (Figure 4). The trophic profile of *C.*

gilchristi overlaps completely with *P. knysnaensis* and *G. callidus*, with this species potentially a generalist. *Glossogobius callidus* is predicted to be better at predating on larger prey (such as fish) and *P. knysnaensis* to be better at predating on small prey (phytoplankton and zooplankton), sessile algae and detritus (Figure 4).

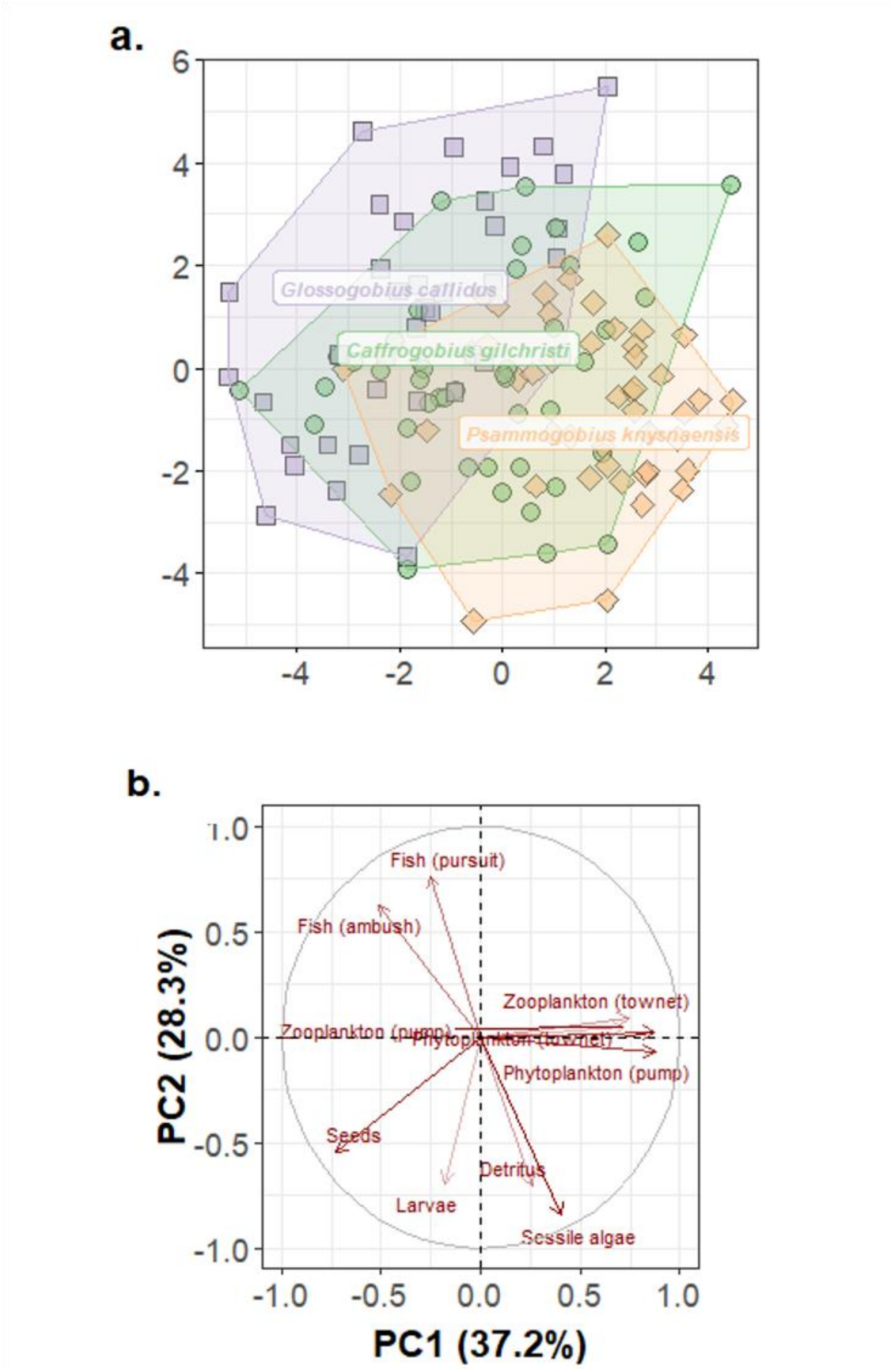


Figure 4: Principal component analysis of the trophic profiles of *Psammogobius knysnaensis, Caffrogobius gilchristi* and *Glossogobius callidus* collected from the Sunday Estuary, South Africa. A different colours represent different species and each marker represents an individual. B represents the sizes of the loading and directions of food specialist profiles

Diet

In total, 273 foreguts were examined for *P. knysnaensis*. Of the foregut 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 and Table 1). Diet composition significantly changed ($\chi^2 = 377.8$, df = 48, $p < 0.05$) with fish size (Figure 5), with an ontogenetic shift in diet occurring from 30–39 mm TL, with ostracods and cyclopods together comprising less than 50% of the diet (<50% IRI) (Figure 5). In individuals of 40–49 mm TL, bivalves contributed 52.88% IRI. This prey item was only recorded in the foregut of individuals larger than 30 mm. Amphipod consumption increased with fish size, with amphipods recorded in the largest numbers in the largest size class (50-59 mm TL) (%IRI = 31.26%). Chironomids (%IRI = 3.08%) were also most prevalent in the diet of the largest size class (Figure 5).

A total of 150 foreguts of *C. gilchristi* were analysed. Of all the foreguts analysed, only one was empty. A total of 12 prey taxa were identified in the diet of *C. gilchristi* (Figure 5 and Table 1), with Cyclopoida having the highest contribution (20–49 mm = > 70%; > 49 mm = > 20%) to this species' diet both in terms of %IRI and %N. Prey consumption changed significantly with fish size ($\chi^2 = 165.4$, df = 33, $p < 0.05$). An ontogenetic shift in diet occurred from 50 mm TL, when cyclopods comprised less than 50% of the diet (<50% IRI) (Figure 5 and Table 1). Gastropoda were only recorded in the foregut 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 = 3.79%) and largest size classes (50–59 mm TL) (IRI = 7.28%). Ostracods, although also consumed by all size classes, comprised a relatively small proportion of the diet in all size classes (0.63 –5.56 %IRI).

A total of 146 foregut of *G. callidus* were analysed. Of the foreguts analysed only one was empty. A total of 10 prey taxa were identified in the diet of this species (Figure 5 and Table 1), with amphipods, chironomids and cyclopoids being dominant in the diet of this species. 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 = 1.23%) and mysid (IRI = 8.74%) consumption was only noticeable in the diet of the largest size class (> 69 mm). The overall diet of the study species is shown in table 1.

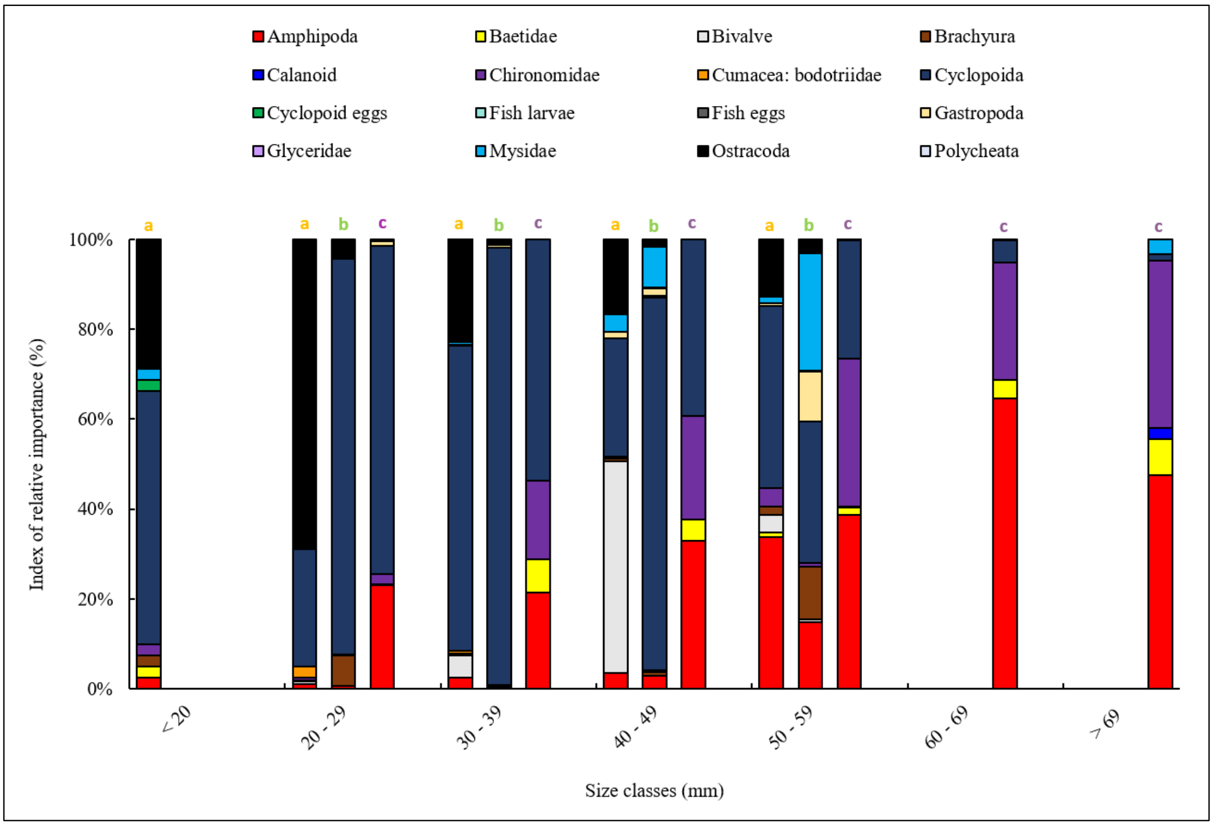
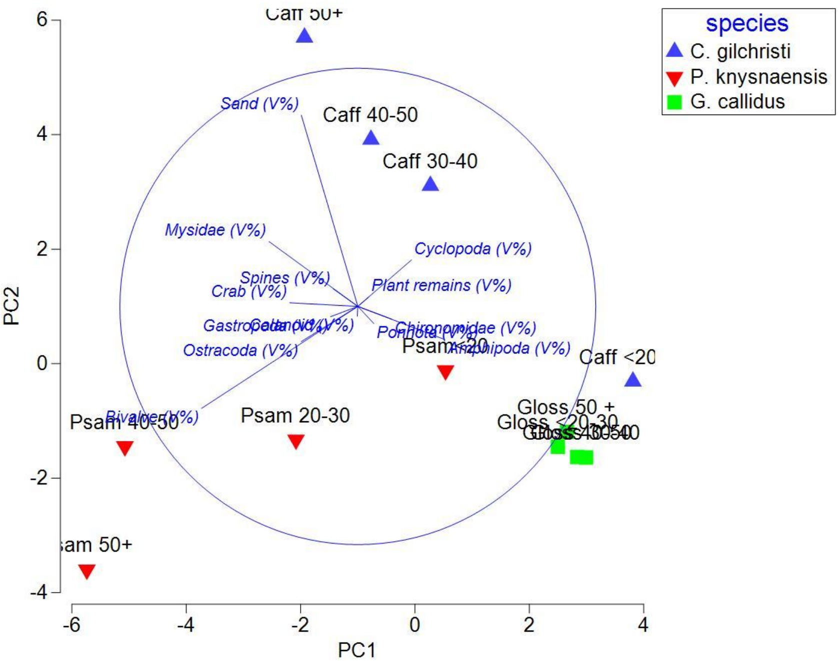


Figure 5: Percentage index of relative importance (%IRI) of prey items found in the foregut of different size classes of *Psammogobius knysnaensis*, *Caffrogobius gilchristi* and *Glossogobius callidus* sampled in September/October 2018 and August 2019 from the Sundays Estuary, Eastern Cape, South Africa. **a** = *Psammogobius knysnaensis*, **b** = *Caffrogobius gilchristi* and **c** = *Glossogobius callidus.*

Table 1: Gut contents of *Psammogobius knysnaensis, Caffrogobius gilchristi* and *Glossogobius callidus* sampled in 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 and %IRI index of relative importance, as a proportion of the total IRI of all species sampled). Bold values indicate high %IRI contribution.

Species	***Psammogobius knysnaensis***		***Caffrogobius gilchristi***		***Glossogobius callidus***	
No of Specimen	276		154		146	
Mean TL (mm)	37,58 (±9,04)		4,98 (±9,45)		44,85 (±14,62)	
Min and Max TL (mm)	17,1-62,2		20,5-94		19,1-83,4	
Prey taxa	**%N**	**%IRI**	**%N**	**%IRI**	**%N**	**% IRI**
Ostracoda	37,1	**30,1**	7,7	**2,4**	2,3	0,1
Cyclopoida	36,5	**49,6**	67,4	**92,3**	47,0	**42,8**
Chironomidae	1,0	0,5	1,2	0,1	17,1	**18,9**
Amphipoda	5,0	3,5	7,9	**2,1**	25,4	**34,3**
Cyclopoid eggs	2,3	0,1	-	-	-	-
Mysidae	1,2	1,5	2,9	1,0	0,4	0,1
Baetidae	0,2	<0.1	-	-	5,7	3,6
Brachyura	0,7	0,4	1,8	0,5	0,2	<0.1
Fish eggs	-	-	3,6	0,1	-	-
Bivalve	13,0	**13,3**	0,1	<0.1	-	-
Cumacea: bodotriidae	1,1	0,6	0,2	<0.1	-	-
Gastropoda	1,1	0,4	6,6	1,4	1,1	0,1
Fish larvae	0,1	<0.1	-	-	0,1	<0.1
Polychaeta	0,5	0,1	0,1	<0.1	-	-
Glyceridae	-	-	0,4	<0.1	-	-
Calanoid	0,3	<0.1	-	-	0,8	<0.1

Niche breadth was highest in for *G. callidus*, (0.35) followed by *P. knysnaensis* (0.30) and *C. gilchristi* (0.27).



Some of the food items predicted to be present in the diet of the study species have been proven to be true in their dietary analysis (Figure 10). Detritus, zooplankton and larvae are present in the diet of *P. knysnaensis*. Crustaceans, molluscs, zooplankton and larvae are also recorded in the diet of *C. gilchristi*. Similarly, insects and mollusc were present in the diet of *G. callidus*.

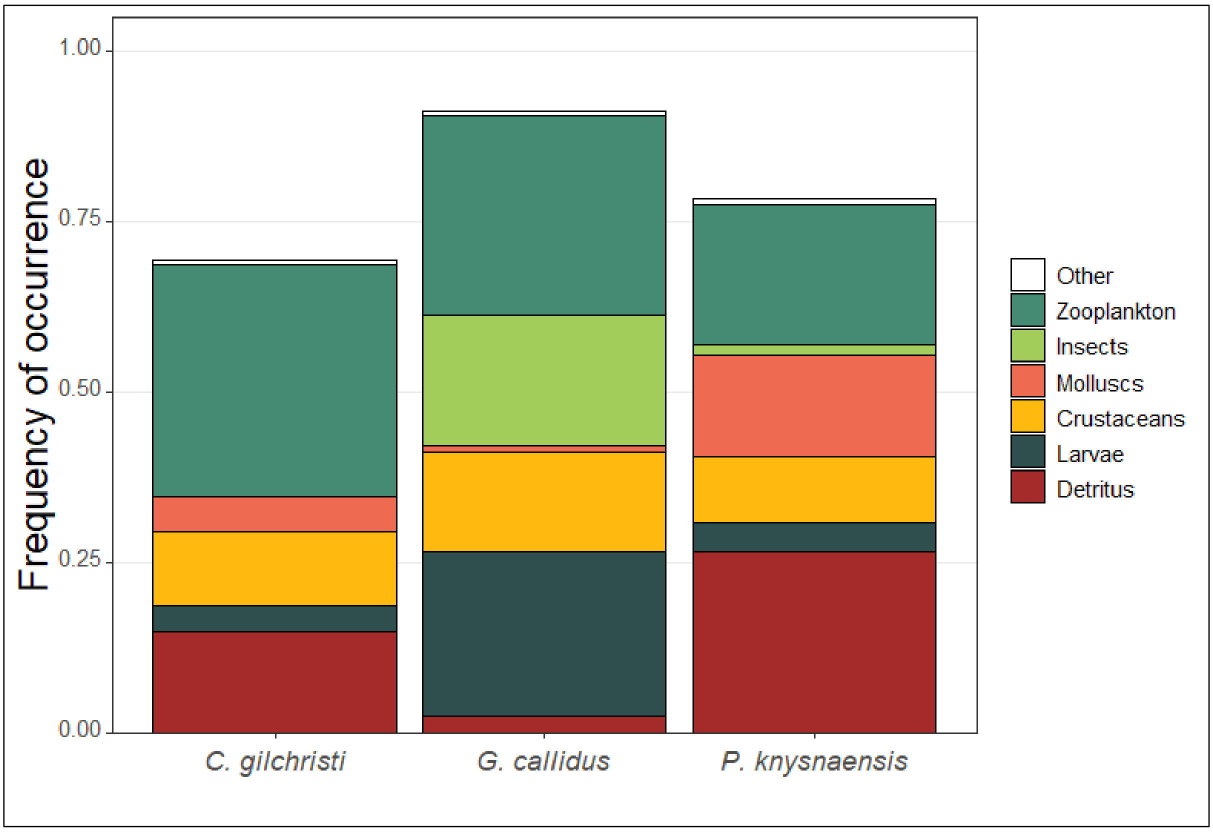


Figure 6: Frequency of occurrence of food fish model (FFM) food types in the diet of *Psammogobius knysnaensis*, *Caffrogobius gilchristi* and *Glossogobius callidus* from the Sundays River Estuary, Eastern Cape, South Africa.

Prey resources

Here please can you plot density of the major prey taxa at each site. You can do this as a stacked bar graph.

So these groups, not species per group:

🟥 Amphipoda
🟨 Baetidae
⬜ Bivalve
🟫 Brachyura
🟦 Calanoid
🟪 Chironomidae
🟧 Cumacea: bodotriidae
⬛ Cyclopoida
🟩 Cyclopoid eggs
⬜ Fish larvae
⬛ Fish eggs
🟨 Gastropoda
🟪 Glyceridae
🟦 Mysidae
⬛ Ostracoda
⬜ Polycheata

DISCUSSION

The morphological traits and trophic profiles show high overlap between *C. gilchristi* and *G. callidus* suggesting high dietary overlap between the two species. This was proven to be true as both species feed on cyclopoids and amphipods. Morphological overlap was also observed in the diet of all study species as the cyclopoids were common during small sizes and the incorporation of amphipods and chironomids increased with fish size.

The overlap in the feeding morphology resulted to the dietary overlap between *G. callidus* and *P. kysnaensis* in the upper reaches of the estuary. Both species had high consumption of cyclopoids and amphipods among the small size classes, with only amphipods common in the diet of the large sizes. These two species had a more similar dietary niche among both small and large sizes than predicted in the morphological analysis. 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.

Psammogobius 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. This means that *G. callidus* potentially outcompetes *P. knysnaensis* in the upper reaches explaining why they dominated in different habitats. 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.* Prey availability in the feeding environments may have caused the differences in the diet of the species. 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).

Glossogobius callidus is 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 (Ndaleni 2022, Wasserman, 2012). 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).

In terms of both morphological traits and trophic profiles, *C. gilchristi* and *P. kysnaensis* are fairly distinct from each other. This resulted to different diet between *C. gilchristi* and *P. kysnaensis* in the lower section, 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).

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, 1997; 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. 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 (Ndaleni 2022). 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. 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.*

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, pharyngeal jaw symbiosis and caudal peduncle depth. 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). 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.

CONCLUSION

The three study species partitioned their resources by occupying different habitats. Prey availability, together with sediment characteristics and temperature, promoted habitat partitioning. 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.

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Table 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. (2018). Numbers represent traits labels in Figure 2. Descriptions of the traits measured are only provided for those not clearly illustrated in Figure 2.

	Morphological trait	**Abbreviation**	**Description**	**Unit**	**Figure 2**
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)	1 - 5	
	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	

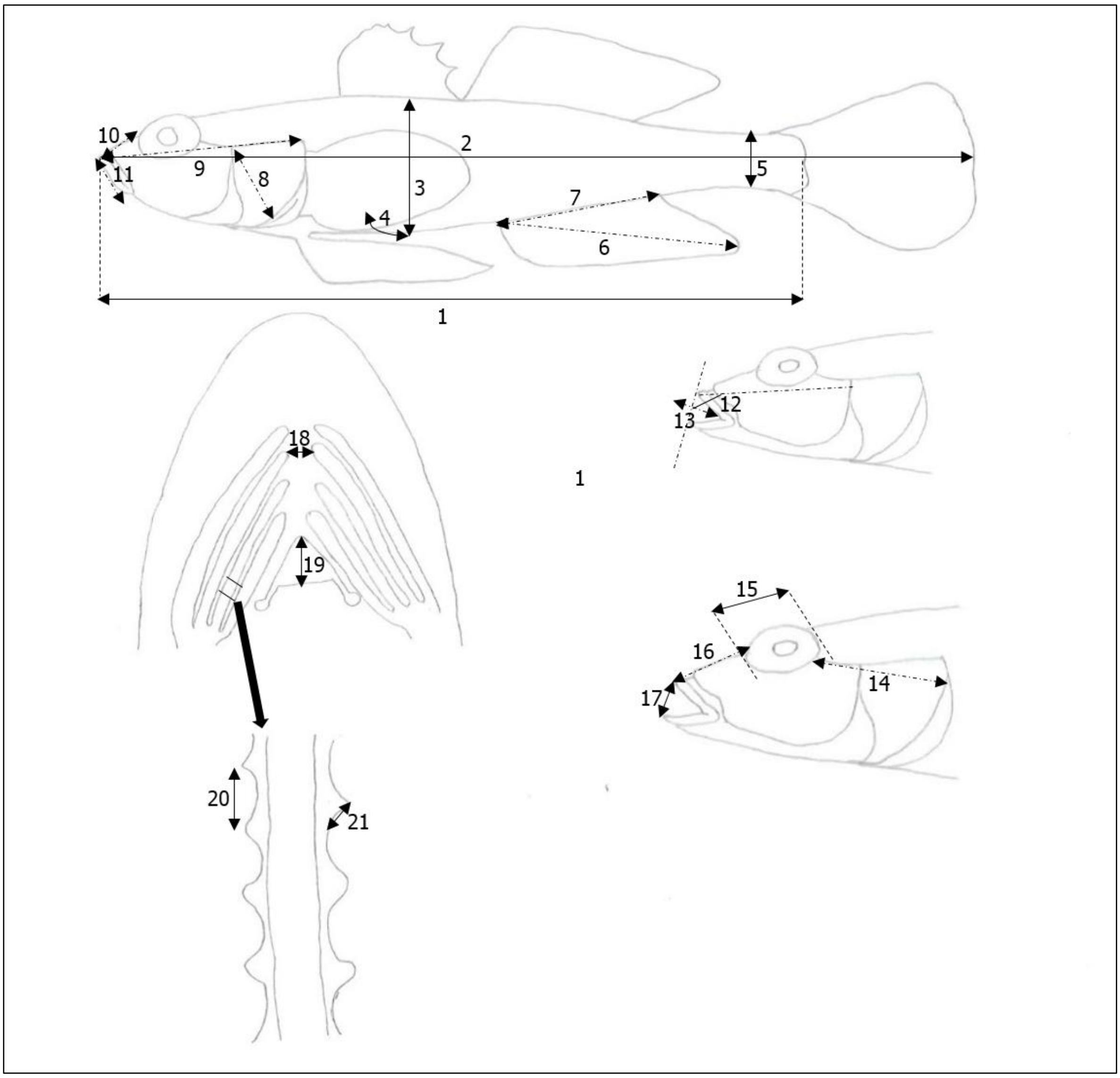

Figure 2: Illustration of the morphological traits measured on *Psammogobius knysnaensis*, *Caffrogobius gilchristi* and *Glossogobius callidus* collected from the Sundays Estuary in July 2018, using *P. knysnaensis* as an example. Internal head measurements are not illustrated as their accurate drawing requires an X-ray scanning of the fish head. Different numbers represent measured traits with descriptions provided in Table 2.

Table 2: 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 foregut containing prey, %V is the volume of each prey item consumed, as a percentage of the total volume of foregut 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***					
Size	**n**	**Prey Taxa**	**n 2**	**%N**	**%F**	**%V**	**IRI**	**% IRI**	**n**	**n 2**	**%N**	**%F**	**%V**	**IRI**	**% IR**
<20	5	Ostracoda	9	32,14	40,00	14,71	1873,95	28,72		-	-	-	-	-	-
		Cyclopoida	7	25,00	60,00	14,71	2382,35	36,51		-	-	-	-	-	-
		Chironomidae	2	7,14	20,00	11,76	378,15	5,80		-	-	-	-	-	-
		Amphipoda	2	7,14	20,00	11,76	378,15	5,80		-	-	-	-	-	-
		Cyclopoid eggs	2	7,14	20,00	11,76	378,15	5,80		-	-	-	-	-	-
		Mysidae	2	7,14	20,00	11,76	378,15	5,80		-	-	-	-	-	-
		Baetidae	2	7,14	20,00	11,76	378,15	5,80		-	-	-	-	-	-
		Brachyura	2	7,14	20,00	11,76	378,15	5,80		-	-	-	-	-	-
20-29	50	Ostracoda	211	57,97	42,00	16,67	3134,62	60,92	17	33	20,75	23,53	7,89	674,10	5,5
		Cyclopoida	119	32,69	26,00	30,10	1632,59	31,73		116	72,96	76,47	69,74	10911,80	89,
		Chironomidae	4	1,10	6,00	3,48	27,49	0,53		1	0,63	5,88	2,63	19,18	0,1
		Amphipoda	8	2,20	8,00	6,22	67,33	1,31		3	1,89	11,76	3,95	68,64	0,5
		Mysidae	2	0,55	2,00	22,89	46,87	0,91		-	-	-	-	-	-
		Baetidae	-	-	-	-	-	-		-	-	-	-	-	-
		Brachyura	1	0,27	2,00	1,49	3,53	0,07		6	3,77	23,53	15,79	460,31	3,7
		Bivalve	4	1,10	6,00	7,96	54,35	1,06		-	-	-	-	-	-
		Cumacea: bodotriidae	14	3,85	12,00	10,95	177,50	3,45		-	-	-	-	-	-
		Gastropoda	1	0,27	2,00	0,25	1,05	0,02		-	-	-	-	-	-
30-39	112	Ostracoda	518	34,51	24,11	6,25	982,57	18,91	48	22	4,56	12,50	3,43	99,91	0,6
		Cyclopoida	656	43,70	42,86	38,39	3518,11	67,70		426	88,38	83,33	85,43	14484,24	98,
		Chironomidae	7	0,47	4,46	0,61	4,80	0,09		-	-	-	-	-	-
		Amphipoda	58	3,86	11,61	6,95	125,55	2,42		14	2,90	6,25	2,71	35,12	0,2
		Cyclopoid eggs	87	5,80	1,79	0,29	10,87	0,21		-	-	-	-	-	-
		Mysidae	8	0,53	5,36	14,48	80,44	1,55		1	0,21	2,08	3,43	7,58	0,0
		Baetidae	-	-	-	-	-	-		-	-	-	-	-	-
		Brachyura	9	0,60	5,36	1,19	9,56	0,18		5	1,04	10,42	3,57	48,01	0,3
		Bivalve	110	7,33	13,39	24,74	429,43	8,26		-	-	-	-	-	-
		Cumacea: bodotriidae	20	1,33	6,25	2,50	23,95	0,46		-	-	-	-	-	-
		Gastropoda	8	0,53	1,79	0,64	2,10	0,04		14	2,90	8,33	1,43	36,11	0,2
		Fish larvae	1	0,07	0,89	1,41	1,32	0,03		-	-	-	-	-	-
		Polychaeta	9	0,60	2,68	1,92	6,76	0,13		-	-	-	-	-	-
		Calanoid	10	0,67	0,89	0,64	1,17	0,02		-	-	-	-	-	-
40-49	81	Ostracoda	623	37,99	20,99	7,71	959,16	15,37	71	48	6,08	15,49	1,20	112,82	1,6
		Cyclopoida	476	29,02	32,10	13,05	1350,54	21,65		486	61,60	57,75	26,87	5108,91	72,
		Chironomidae	9	0,55	6,17	0,51	6,51	0,10		16	2,03	7,04	2,60	32,60	0,4
		Amphipoda	67	4,09	16,05	5,92	160,60	2,57		64	8,11	14,08	9,24	244,44	3,4
		Mysidae	31	1,89	17,28	19,56	370,67	5,94		39	4,94	23,94	53,68	1403,65	19,
		Baetidae	1	0,06	1,23	0,23	0,36	0,01		-	-	-	-	-	-
		Brachyura	12	0,73	8,64	1,34	17,94	0,29		11	1,39	9,86	1,78	31,30	0,4
		Fish eggs	-	-	-	-	-	-		58	7,35	2,82	1,51	24,95	0,3
		Bivalve	376	22,93	46,91	47,39	3298,81	52,88		-	-	-	-	-	-
		Cumacea: bodotriidae	8	0,49	6,17	0,66	7,10	0,11		4	0,51	1,41	0,55	1,49	0,0
		Gastropoda	28	1,71	18,52	1,75	64,08	1,03		58	7,35	11,27	1,68	101,73	1,4
		Fish larvae	1	0,06	1,23	0,86	1,13	0,02		-	-	-	-	-	-
		Polychaeta	8	0,49	1,23	1,01	1,85	0,03		1	0,13	1,41	0,41	0,76	0,0
		Glyceridae	-	-	-	-	-	-		4	0,51	5,63	0,48	5,56	0,0
		Calanoid	-	-	-	-	-	-		-	-	-	-	-	-
50-59	28	Ostracoda	103	29,86	25,00	5,81	891,51	14,28	18	22	11,22	11,11	3,87	167,76	3,5
		Cyclopoida	121	35,07	50,00	13,23	2415,23	38,69		69	35,20	27,78	10,58	1271,67	26,
		Chironomidae	12	3,48	28,57	3,24	192,03	3,08		3	1,53	11,11	2,93	49,58	1,0

		Amphipoda	64	18,55	42,86	26,99	1951,64	31,26		47	23,98	16,67	15,50	657,95	13,
		Mysidae	4	1,16	10,71	21,76	245,54	3,93		7	3,57	33,33	54,45	1934,06	40,
		Baetidae	3	0,87	10,71	2,20	32,85	0,53		-	-	-	-	-	-
		Brachyura	5	1,45	10,71	2,30	40,18	0,64		8	4,08	27,78	8,48	348,98	7,2
		Bivalve	25	7,25	14,29	23,22	435,26	6,97		1	0,51	5,56	1,68	12,14	0,2
		Gastropoda	8	2,32	10,71	1,26	38,29	0,61		36	18,37	16,67	2,09	341,03	7,1
		Fish larvae	-	-	-	-	-	-		-	-	-	-	-	-
		Glyceridae	-	-	-	-	-	-		3	1,53	5,56	0,42	10,83	0,2
		Calanoid	-	-	-	-	-	-		-	-	-	-	-	-
60-69		Cyclopoida	-	-	-	-	-	-		-	-	-	-	-	-
		Chironomidae	-	-	-	-	-	-		-	-	-	-	-	-
		Amphipoda	-	-	-	-	-	-		-	-	-	-	-	-
		Mysidae	-	-	-	-	-	-		-	-	-	-	-	-
		Baetidae	-	-	-	-	-	-		-	-	-	-	-	-
		Brachyura	-	-	-	-	-	-		-	-	-	-	-	-
>70		Cyclopoida	-	-	-	-	-	-		-	-	-	-	-	-
		Chironomidae	-	-	-	-	-	-		-	-	-	-	-	-
		Amphipoda	-	-	-	-	-	-		-	-	-	-	-	-
		Mysidae	-	-	-	-	-	-		-	-	-	-	-	-
		Baetidae	-	-	-	-	-	-		-	-	-	-	-	-
		Calanoid	-	-	-	-	-	-		-	-	-	-	-	-