BIOLOGY AND ECOLOGY OF *GLOSSOGOBIUS CALLIDUS* (SMITH 1937) IN IRRIGATION IMPOUNDMENTS IN THE SUNDAYS RIVER VALLEY OF THE EASTERN CAPE

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The biology and ecology of *Glossogobius callidus* (Smith 1837) in irrigation impoundments in the Sundays River Valley, Eastern Cape, South Africa

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GENERAL ABSTRACT

The River Goby *Glossogobius callidus* (Smith, 1937) is a native abundant fish in both freshwater and estuarine habitats in the Cape Fold Ecoregion, yet little information is available on its life-history. This study aims to contribute to knowledge on the age and growth, reproductive biology and the diet and feeding habits of *G. callidus* in irrigation impoundments.

Glossogobius callidus was sampled monthly from August 2013 till March 2015; from the irrigation ponds in the Sundays River Valley, Eastern Cape Province, South Africa. To determine sex, gonads were visually assessed under a dissecting microscope to confirm the sex based on the shape of the urogenital papillae. Fish were then dissected to confirm sex and gonads were categorised into five macroscopic stages which were histologically validated. Microscopic stages of gonadal development were discerned based on nuclear and cytoplasmic characteristics of the oocyte or sperm. Ovaries and sperms were assigned stages based on the most advanced type of oocyte present. In total 2054 fishes ranging in length from 21.1 mm to 137.2 mm TL were sampled. The sex ratio (1.1 males: 1 females) did not differ from unity ($x^2 = 0.027$, $df = 1$, $p = 0.87$). Length at 50% maturity (L_m) was 70 mm TL females and 72 mm TL for males. Spawning season was mid-spring and mid-summer and mean ± S.D absolute fecundity was estimated at 1028.2 ± 131.7 ova/fish. Relative fecundity (number of vitellogenic oocytes per gram of eviscerated fish mass) were estimated at 50 ± 18 ova/fish gram.

Otoliths from 560 fish were used for ageing. Growth zone deposition rate was validated using edge analysis. As a unimodal periodic regression model best described the temporal proportion of opaque zone deposition on the edge of otoliths over a one-year period, growth zone deposition rate was validated as annual. The oldest female fish was a 4-year old 84.4 mm TL fish and the oldest male was a 7-year old 100.5 mm TL fish. The length-at-age for the entire population of 560 *G. callidus* provided von Bertalanffy parameters of $L_t = 92\,(1 - e^{-0.58(t + 0.4)})$ mm TL for the entire population, $L_t = 70\,(1 - e^{-1.8\,(t + 0.06)})$ mm TL for males and $L_t = 65\,(1 - e^{-1.8\,(t + 0.05)})$ mm TL for females. Converting length at maturity to age at maturity demonstrated that *G. callidus* attained maturity at an age of 2-years. Growth performance described using the phi-prime index showed that *G. callidus* had lower growth performance compared to the invasive *Neogobius melanostomus*. Using age structure, natural mortality was estimated at 1.31 yr^{-1} using catch curve analysis. Diet of *G. callidus* comprised of ten taxonomic groups. Among these, aquatic invertebrates were the most diverse group but while relative contribution of the dietary components varied across all size classes and seasons, the key prey items were consistently found in all size classes. These were Diptera, Hemiptera, Trichoptera, Odonata, Cladocera, Copepoda, Hydracarina, Amphipoda, Crustacea, and Mollusca. While dietary differences were observed between the size classes and throughout the seasons, *G. callidus* can be regarded as a generalist feeder preying on an array of different species. Given its abundance and diet, I suggest that *G. callidus* contribute considerably to the invertebrate predation pressure in these artificial aquatic environments in an arid region.

In summary, medium fecundity, fast growth, moderate maturity, and a generalist feeding behaviour demonstrate that *G. callidus* is an equilibrium life strategist. In comparison with other species, the life-history traits of *G. callidus* from irrigation impoundments resemble those of other freshwater goby species, some of which are global invaders.

Key words: life-history traits, reproductive biology, age and growth, feeding ecology and equilibrium strategist.

TABLE OF CONTENTS

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CHAPTER 1

GENERAL INTRODUCTION

The increase in human populations and inadequate access to, or appropriate management of, freshwater supply has resulted in a worldwide crisis (Glieck, 1998). Satisfying a thirsty world and conserving biological diversity remains a daunting task (Grant *et al*., 2012). This is especially concerning as human activities have impacted heavily on biodiversity and biological resources especially for inland waters (Dudgeon *et al*., 2006). Among the many threats to global freshwater biodiversity, overexploitation; water pollution; flow modification; destruction or degradation of habitat; and invasion by alien species are the most pertinent (Dudgeon *et al*., 2006)

Freshwater ecosystems are at the forefront of a major crisis and the construction of impoundments alters habitat structure and connectivity in riverine ecosystems (Johnson *et al*., 2008). Native species often do not adapt to these changes and as a result, impoundments are often colonized by non-native species and, therefore, have often facilitated biological invasions (Johnson *et al*., 2008). Therefore understanding the biological traits that facilitate colonization of these novel habitats by native and non-native fishes is important.

An opportunity to study the biology of fishes colonizing novel environments is presented by the Sundays River Irrigation Scheme (SRIS), which is part of the Orange-Fish-Sundays inter basin water transfer (IBWT) scheme that was completed in 1975 to allow for large-scale irrigation farming in the Great Fish and Sundays River Valley (Laurenson and Hocutt, 1984). The IBWT transfers a maximum of 1.7 x 109 m^3 of water primarily for irrigation from the Orange River Basin to the Great Fish and Sundays rivers. This IBWT facilitated the passive introduction of several freshwater fishes which are native to the Orange-Vaal system but alien to the Sundays River system (e.g., Orange River Mudfish *Labeo capensis* and the Smallmouth Yellowish *Labeobarbus aeneus* and African Sharptooth Catfish *Clarias gariepinus*; Cambray and Jubb, 1977; Laurenson and Hocutt, 1984; Meador, 1992; Snaddon *et al*., 1998). In addition, the IBWT also facilitated the spread of native such as (e.g., Gilchrist`s Round Herring *Gilchristella aestuaria*, River Goby *Glossogobius callidus*, Moggel *Labeo umbratus*, African Longfin Eel *Anguilla mossambica* and alien (e.g., Mosquitofish *Gambusia affinis*, Mozambique Tilapia *Oreochromis mossambicus* and Common Carp *Cyprinus carpio*, through a complex irrigation network to hundreds of small irrigation ponds used as reservoirs to irrigate citrus orchards (Howell *et al*., 2013).

Glossogobius callidus, *G. aestuaria*, *G. affinis*, and *O. mossambicus* were the four most successful species in establishing in the ponds, partly because of propagule pressure originating from the irrigation network and partly due to the reproductive strategies of the individual species (Woodford *et al*., 2013). According to Woodford *et al*. (2013) the native River Goby *G. callidus* (Smith, 1937) were the most abundant species entering the network and its densities were positively correlated with propagule pressure. They further pointed out that *G. callidus* was the fastest establishing species within the network and this could have been facilitated by suitable reproductive, environmental, and feeding traits.

Understanding these traits remains one of the greatest challenges and requires detailed knowledge of the species natural life history (Angert *et al*., 2011). According to Balon (1981a) life-history styles are consequences of adaptation to environmental conditions and understanding these styles can reveal the life history and population dynamics of the species of interest. Life-history styles often depend on environmental variability (Winemiller, 2005). Fishes that occur in stable environments for example, often exhibit traits associated with a precocial lifestyle (e.g., delayed maturity, larger size, low fecundity per spawning event, and high juvenile survivorship (i.e., greater parental care)) (Balon, 1981a), while fishes inhabiting unstable environments tend towards atricial life-history styles typified by early maturity, high fecundity, and small body size (Balon, 1981b). In an attempt to develop a framework for characterising the reproductive styles of fishes, Winemiller and Rose (1992) used aspects of the life-history of fishes to characterise them as periodic, equilibrium, and opportunistic strategists (Figure 1.1).

There has been considerable interest in identifying major drivers of ecological variation in freshwater ecosystems (Olden and Kennard, 2010). Most research has been primarily based on correlations between biological traits and the environment across large numbers of species (Olden *et al*., 2006; Winemiller, 2005). These assessments have shown that species traits are inter-correlated through physiological constraints, trade-offs, or spin-offs resulting in the evolution of life history strategies or tactics represented as sets of coevolved traits that enable a species to cope with a range of ecological problems (Winemiller and Rose, 1992). The triangular continuum (see Figure 1.1) proposed by Winemiller and Rose (1992) was from comparative studies arising from essential trade-offs among demographic parameters of survival, fecundity and onset and duration of reproduction from studying an array of fishes in both marine and freshwater systems.

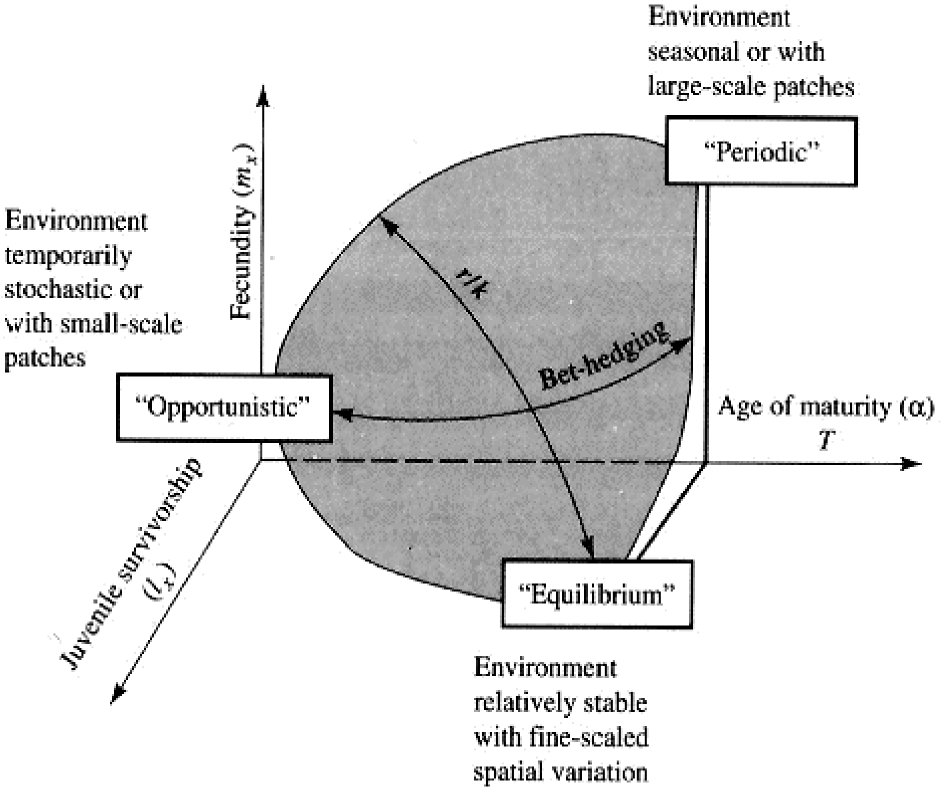

Figure 1.1: Three endpoint life-histories for fishes derived by Winemiller and Rose (1992). The endpoint strategies are opportunistic, equilibrium and periodic.

Biological characteristics and habitat environmental attributes are associated with each strategy: (i) periodic strategists are large-bodied fishes with delayed maturity, high fecundity per spawning season, are associated with low juvenile survivorship (i.e., no parental care) and typically inhabit seasonal, periodically suitable environments; (ii) opportunistic strategists are small-bodied fishes inhabiting highly disturbed and unpredicted environments with early maturation, low fecundity per spawning season event, and low juvenile survivorship; (iii) equilibrium strategists are small- to medium-bodied fishes with moderate maturation age, low fecundity and high juvenile survivorship (i.e., greater parental care) that typically inhabit constant environments.

According to Winemiller (2005), the Winemiller and Rose (1992) framework can be viewed as an adaptive suite of attributes with respect to relative intensity and predictability of temporal and spatial variation in abiotic environmental conditions, food availability and predator pressure. This model provides core structure in understanding and predicting fish population responses to changing environments (Olden *et al.*, 2006) and as a result, research on the understanding life history of fishes needs to investigate the principal components: fecundity, maturity, age, longevity, mortality and reproductive style. In this thesis, I contribute to better understanding of these processes by undertaking a detailed ecological study on one of the successful colonizers of the SRIS, the freshwater goby *Glossogobius callidus*.

Study species

The family Gobiidae is one of the largest vertebrate groups that comprises 212 genera and approximately 2000 species worldwide (Thacker, 2003). This fish family is distributed throughout the tropical, subtropical and temperate regions of the world and occurs in freshwater, estuarine and marine habitats (Thacker, 2009). Members of the family are typically small fishes (< 50 cm TL) that are characterized by a fused pelvic fin that, when well-developed, forms an adhesive disc/sucking disc (Nelson, 1994). Gobies are also characterized by their broad and depressed head with a terminal or slightly projected mouth and eyes positioned on top of short stalks capable of being elevated or retracted (Nelson, 1994). While this enormous group of fish species includes nektonic reef-dwellers, planktonic species, and estuarine representatives with the ability to breathe air, the majority of gobies are benthic (Thacker, 2003). The reproductive guild of members of the family Gobiidae is categorized as guarders (Nelson, 1994) that mostly live in close association, spawning in pairs with the eggs deposited in a sheltered site or in bottom burrows (Heemstra and Heemstra, 2004).

Several Ponto-Caspian species belonging to the family are successful colonizers of natural and artificial habitats in Europe and North America (MacInnis and Corkum, 2000a). *Neogobius melanostomus* is a bottom dwelling, aggressive and multi spawning fish that is native to the Ponto-Caspian region of Romania (Siminović *et al.*, 2001) that was introduced to the Great Lakes in North America via blast waters from transoceanic vessels (Kornis *et al.*, 2012). Ever since its introduction, *N. melanostomus* has been one of the most rapidly colonizing species extending its distribution range both in Europe and in the Great Lake of North America (Grula *et al.*, 2012; Phillips *et al.*, 2003). Part of this success is linked to its reproductive strategy that allows for multiple spawning (3 – 4 times a week) over a season (Kornis *et al.*, 2012; MacInnis and Corkum, 2000b). Similarly, in Australia, two gobiids *Acentrogobius pflaumii* and

Acanthogobius flavimanus species have established within a year of introduction through ballast water (Lintermans, 2004).

The genus *Glossogobius* is comprised of about 28 species along the Indo-west Pacific region, nine of which occur in South Africa *G. ankaranensis*, *G. biocellatus*, *G. callidus*, *G. filosus*, *G. giuris*, *G. gutum*, *G. kokius*, *G. tenuiformis* and *G. tongarevae*). A review of the known biological traits of the species in this genus is provided in Table 1.1. What is known is that members of the genus are generally small (max 500 mm TL 30 - 400 mm TL), guarding, benthic invertebrate feeders (Table 1.1).

Table 1: Valid species of *Glossogobius* (n=28) according to FISHBASE (www.fishbase.org). FW = Freshwater; MA = Marine; BR = Brackish)

Scientific name	Env.	Distribution	Habitat	Guild	Feeding	Main ref
G. ankaranensis	FW	Africa, Madagascar	Cave restricted	-	-	1
G. aureus	FW; BR	Africa, Asia, Oceania	Sand or gravel bottoms	Guarder, Clutch tender	invertebrates and small fishes	2
G. bellendenensis	FW	Australia		-	-	3
G. bicirrhosus	MA; FW; BR	Asia, Australia, Oceania	Tidal creeks and river mouths	Guarder, Clutch tender	-	4
G. brunnoides	MA; FW; BR	Western pacific	Fast-flowing streams over gravel or rocky bottoms	Guarder, Clutch tender	-	4
G. bulmeri	FW	Asia, Oceania	Rain forest tributaries, gravel and sand bottoms	Guarder, Clutch tender	-	4
G. celebius	MA; FW; BR	Asia, Oceania	Clear streams	-	-	4
G. circumspectus	FW; BR	Asia, Oceania	Brackish waters and river mouths	-	-	5
G. clitellus	FW	Asia, Oceania	-	-	-	4
G. coatesi	FW	Ocenia	Main river channels and smaller tributaries	-	-	4
G. concavifrons	FW	Oceania	Turbid rivers	Guarder, Clutch tender	Insects and crustaceans	4
G. flavipinnis	FW	Asia	-	Guarder	-	6

Table 1.1: **continued**

Scientific name	**Env.**	**Distribution**	**Habitat**	**Guild**	**Feeding**	**Main ref**
G. giuris	MA; FW; BR	Africa to Oceania	Canals, ditches and ponds	Guarder, Clutch tender	-	4
G. hoesei	FW	Asia	Gravel and mud bottoms	Guarder, Clutch tender	-	4
G. illimis	FW	Asia, Oceania	-	-	-	7
G. intermedius	FW	Asia	-	Guarder	-	6
G. kokius	MA; FW; BR	Africa, Indian Ocean	-	-	-	8
G. koragensis	FW	Asia, Oceania	River channels	Guarder, Clutch tender	Small fishes and crustaceans	4
G. matanensis	FW	Asia	-	Guarder	-	6
G. minutes	MA	Indian Ocean	-	-	-	9
G. munroi	FW	Asia and Oceania	-	-	-	7
G. muscorum	FW	Oceania	-	-	-	3
G. obscuripinnis	FW	Asia	-	-	-	10
G. olivaceus	MA and FW	Northwest Pacific	-	-	-	10
G. robertsi	FW	Oceania	-	-	Small fishes and crustaceans	3
G. sparsipapillus	BR	Western Pacific	-	-	Small fishes and crustaceans	11
G. torrentis	FW	Oceania	Fast-flowing streams	-	-	10

1 = Banister (1994); 2 = Allen (1989); 3 = Hoese and Allen (2009); 4 = Allen (1991); 5 = Allen *et al*., (2002); 6 = Kottelat *et al*., (1993); 7 = Hoese and Allen (2011); 8 = Letourneur *et al*., (2004); 9 = Geevarghese and John (1983); 10 = Kottelat (2013); 11 = Rainboth (1996)

The River Goby *Glossogobius callidus* (Smith, 1937) (Figure 1.2), is a moderately small species with a depressed head, an elongated snout, and a tongue joined to the floor of the mouth (Greenwood, 1994). This species has a pointed caudal fin; the nape is without scales (Skelton, 2001; Smith and Heemstra, 2003). The species occurs from the east coast rivers of Mozambique south to the Swartvlei region of the Western Cape (Skelton, 2001; Whitfield, 1998) (Figure 1.3). This species penetrates far inland, especially at the northern limits of its distributional range where a specimen was collected from the Shire River in Malawi (Tweddle, 2007). *Glossogobius callidus* exhibits wide salinity tolerance as it may be found in estuarine and freshwater habitats where it is exposed to widely different environments (Engelbrecht and Mulder, 1999; Maake *et al*., 2013). It is primarily found living in the bottom of the riverine pools, amongst cover such as cobbles or vegetation, and individual fish usually remain motionless on the bottom unless feeding or fleeing (Skelton, 2001).

Figure 1.2: *Glossogobius callidus* from the Sundays River Valley Irrigation ponds in the Eastern Cape, South Africa. Photo by O. Weyl

Genetic research by Maake *et al*., (2013) revealed that populations of *G. callidus* have two reproductively isolated lineages, with lineage 1 representing the true *G. callidus* distributed mainly along warm-temperate regions and lineage 2 representing a cryptic species that is likely to be widespread in tropical and subtropical regions and this shows that population of *G. callidus* in South African are separate (Figure 1.3). Lineage 1 is represented by three geographic groups in the warm-temperate region; from lineage 1 group 2 *G. callidus* is used in this study.

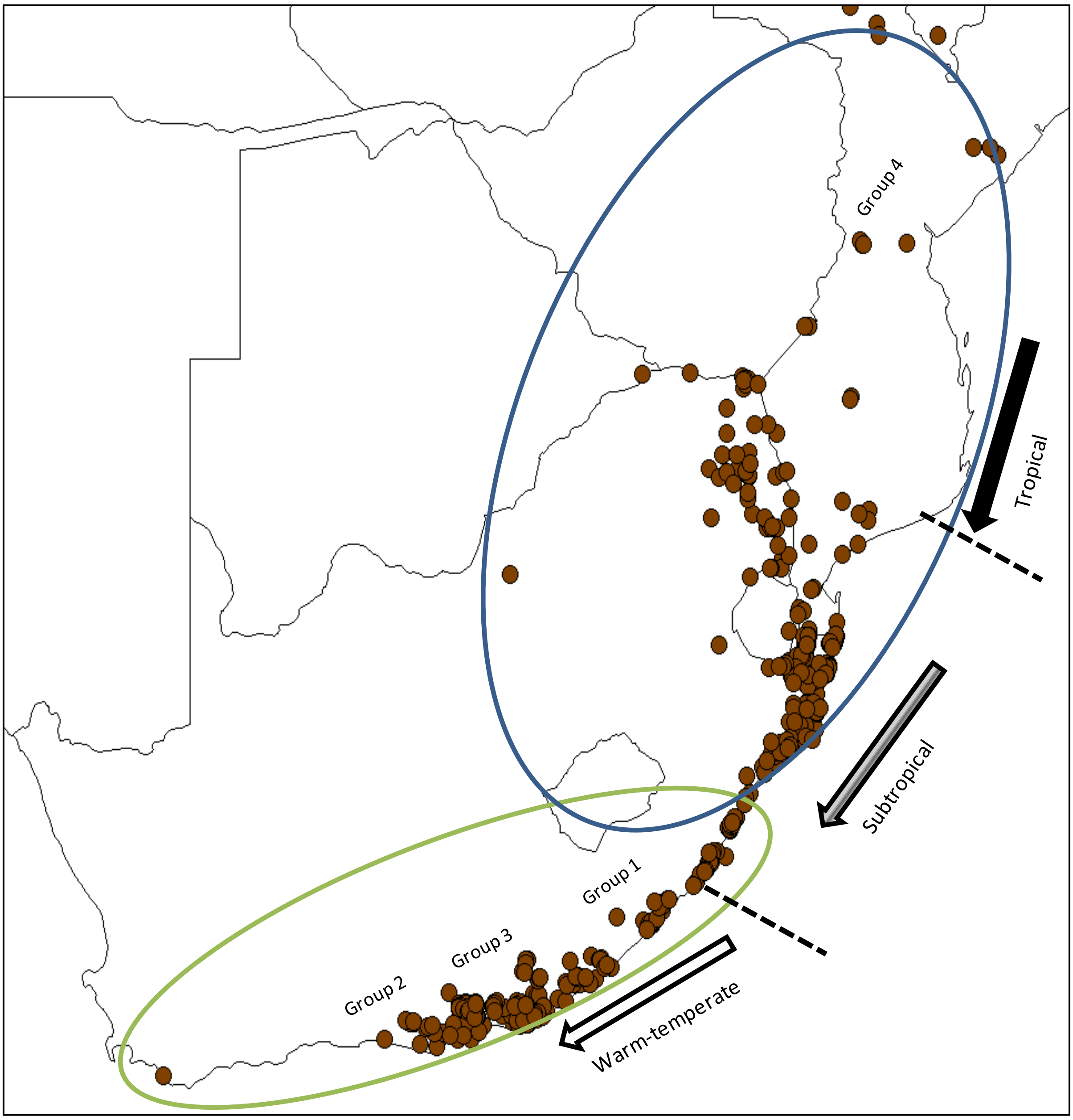

Figure 1.3: Distribution range of *Glossogobius callidus* along the southern African coastline between northern Mozambique to the Cape region of South Africa. Green (lineage 1) and blue circle (lineage 2) illustrate the two different populations, Arrows illustrate the warm-temperate (white arrow), subtropical (grey arrow) and tropical Biogeographic regions of South Africa and Mozambique (Source: SAIAB fish collection records).

Based on a study of the feeding ecology of four small size classes of fish species from a temporarily open/closed estuary, Vumazonke *et al*., (2008) showed that in the estuary dwelling *G. callidus* the main diet comprised of gammarid amphipods and harpacticoid copepods. In freshwaters it is reported that *G. callidus* mostly feeds on the bottom-living insects and small invertebrates (Skelton, 2001). Vumzonke *et al*., (2008) hypothesised that *G. callidus* plays a major role in the ecology of the habitat in which they exist as they are generally abundant and form a major component of the fish biomass and food web (Vumazonke *et al.*, 2008). Despite the abundance of *G. callidus* in both freshwater and estuarine habitats, there are few studies on its biology and ecology. What is documented is that the species has a larval stage which is a dominant component of the estuarine plankton in mesohaline regions near freshwater inflows (Strydom and Neira, 2006). Based on laboratory observations, Wasserman *et al.*, (2015) classified this species reproductive style as speleophilc (hole-nesters) whereby males prepared a nest by inhaling sand particles from inside the nest and expelling them from the nest and subsequently provided parental care, fanning the eggs until hatching.

Thesis structure

This thesis aims to contribute to understanding of the biology and ecology of *G. callidus* in freshwater systems. To do this, important life history traits were determined and compared to those of other goby species to test whether these early colonisers of the irrigation network possess traits similar to other goby species. The general introduction (Chapter 1) is followed by a description of the study area, physical characteristics and general methods used to attain the samples (Chapter 2). Chapter 3 investigates the reproductive biology, which entails maturity, spawning frequency. Growth rate, life-span, mortality are investigated in Chapter 4. Chapter 5 describes the feeding ecology and in the final chapter, (Chapter 6), the overall life history is contextualised.

CHAPTER 2

STUDY SYSTEM

The Sundays River Irrigation Scheme

The Sundays River previously known as the Nqweba River, meaning the river of thorn trees, drains an extensive catchment from Graaff-Reinet to the Indian Ocean just North of Port Elizabeth (Beckley, 1984). The river is approximately 310 km long (Baird, 2001), with the estuary making up approximately 21 km of this length (Scharler and Baird, 2005). The river has a catchment size of 22 063 km^2 and a mean annual runoff of 29 x 106 m^3 (Mackay and Schumann, 1990), with additional water supplied via an inter-basin transfer scheme from the Orange River system (Pech *et al.*, 1995). The Inter-Basin Water Transfer (IBWT) Canal was completed in August 1975; coming from the Orange-Vaal catchment, it then enters the Sunday's system through a Darlington Reservoir tributary. Water released from this 4000 ha impoundment flows 50 km downstream to Korhaansdrift Weir, from where it is diverted into the irrigation network (Pech *et al*., 1995)

A number of in-stream constructions in the main river proved have been shown as complete migration barriers for fishes in the Sunday's main river (Wasserman *et al*., 2011). Thus, Darlington Dam is considered as the invading source for the irrigation network and the irrigation ponds (Woodford *et al*., 2013). From here, the water is transported downstream to Korhaansdrift and the irrigation off-take canal, which directs water away from the river and into the irrigation network of the Sunland district.

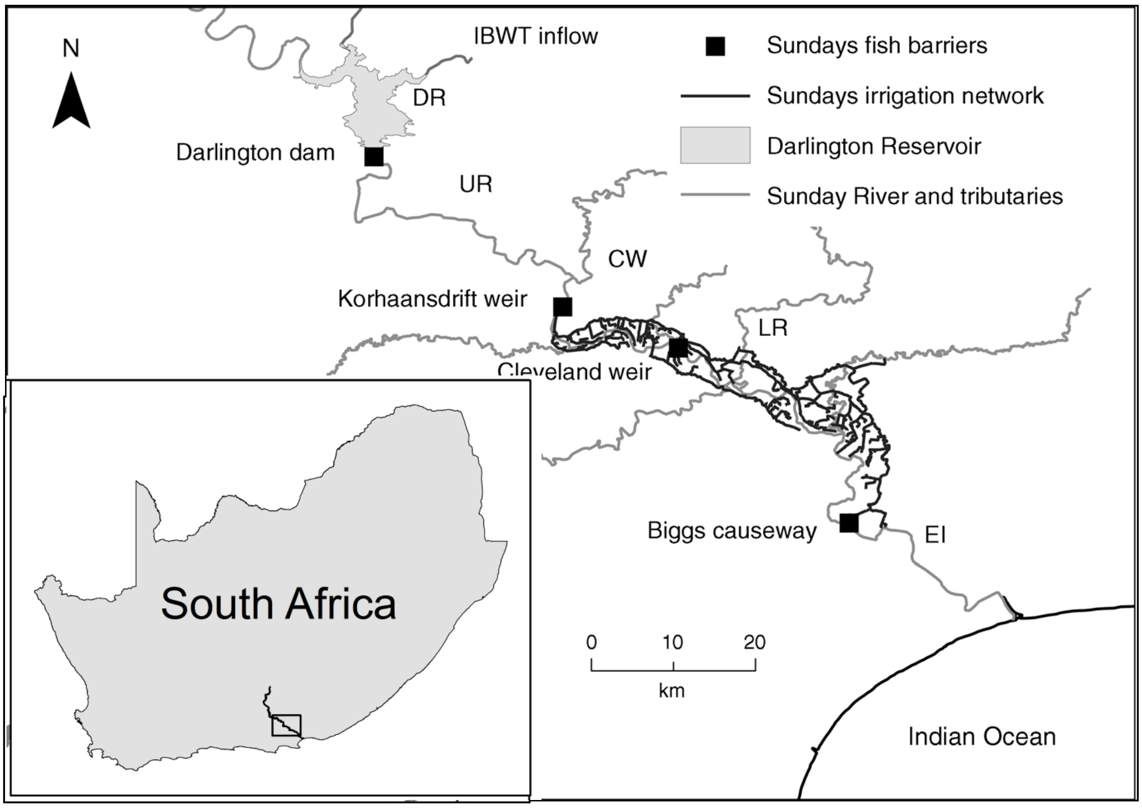

Figure 2.1: Sundays River and its associated tributaries, major barriers on the main stream are labelled in squares. Darlington reservoir (DR); Korhaandrift (UR); Cleveland weir (CW); lower river (LR); and causeway in the estuarine-influenced zone (EI). IBWT represents the inter-basin water transfer. Adapted from Woodford *et al.*, 2013.

Within the network, water flows via gravity down several major and minor concrete canals, eventually feeding about 400 small off-channel irrigation ponds on private farms (Woodford *et al.*, 2013) (Figure 2.1). The Lower Sundays River Water Users Association controls the opening and closing of sluices that connect each pond; this is the same association from which the owners of theses ponds purchase water from on a weekly basis. Each pond receives water up to an annual quota, which is approximately 9000 m^3 per hectare irrigated. Fishes are distributed with the water to the irrigation canals and flushed into the farm ponds.

Each pond has a gravity-fed inflow, where the amount of water purchased by the landowner is introduced at irregular intervals from the canal system. Inflow canals are usually only opened for a few hours per week, therefore, they are dry for the remainder of the week. The passage of fishes out of the ponds is prevented by filtered pipes, which draw water from the ponds whenever it is needed in the farmland (Figure 2.2). Thus, these ponds are dispersal sinks for all introduced fishes.

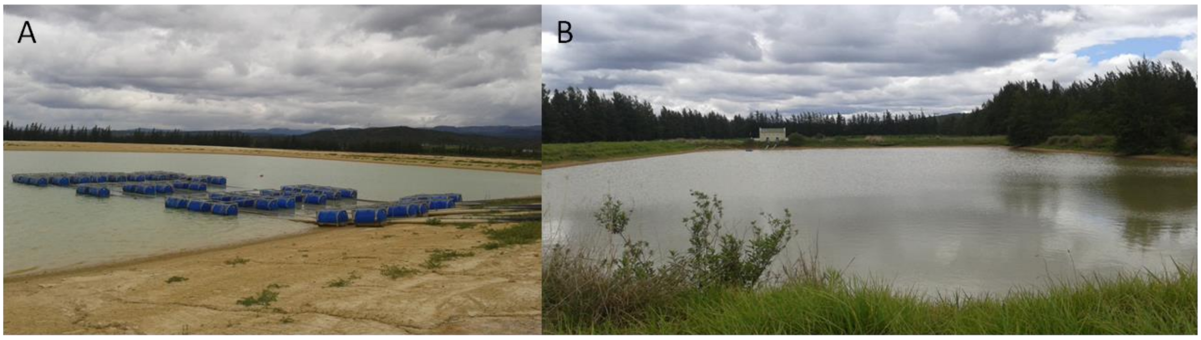

Figure 2.2: (A; B) Irrigation ponds at River Bend Farm in the Sunday River valley, Eastern Cape of South Africa.

Physical characteristics

The Sundays River Valley has a warm-temperate and episodic climate (Kayde and Booth, 2012) with a mean annual temperature of 27.2 °C and a total rainfall fluctuating around 30 mm throughout the year (SAWS, 2015) (Figure 2.3, 2.4).

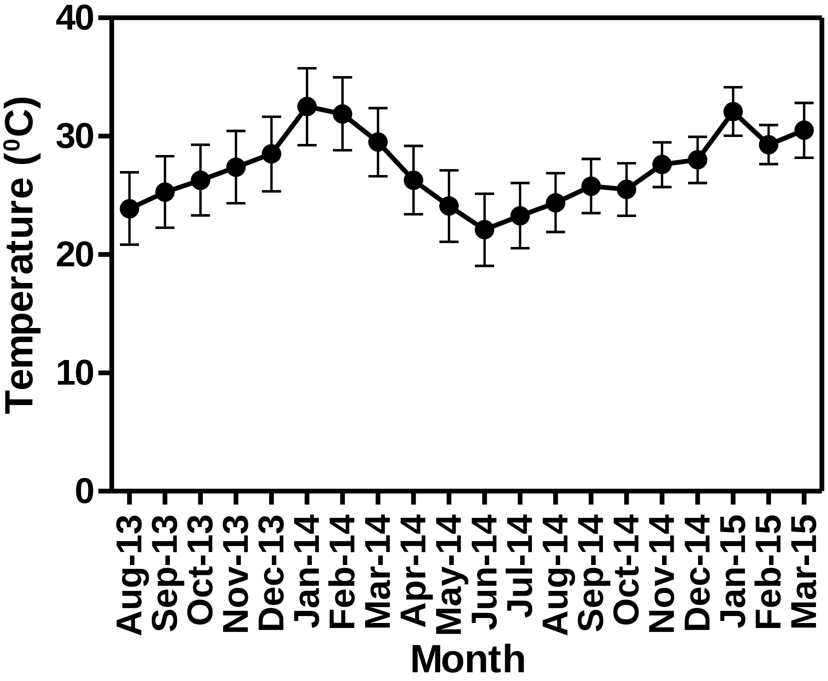

Figure 2.3: Average temperature readings (± SE) from the Sundays River Valley irrigation ponds, Eastern Cape of South Africa. Sourced from the South African Weather Service (SAWS) station in Addo Elephant National Park.

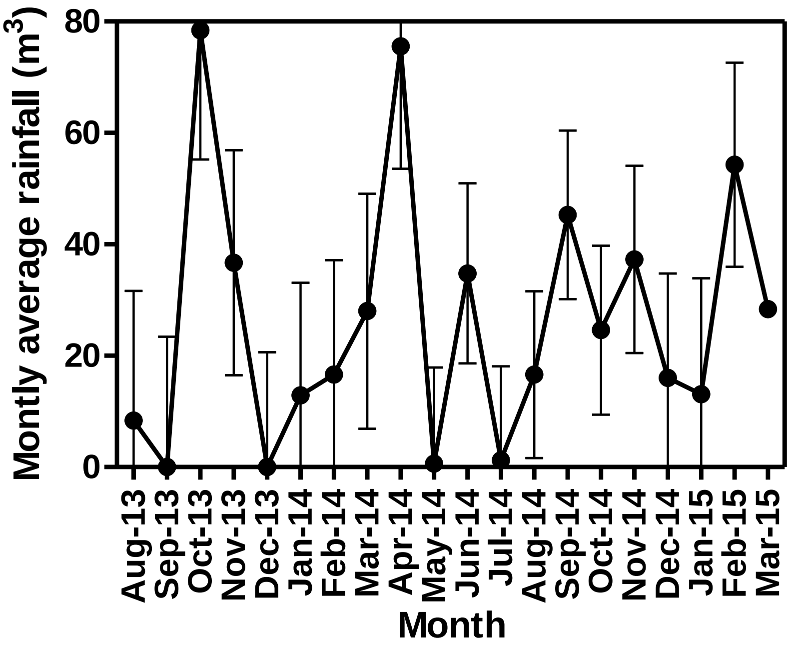

Figure 2.4: Mean monthly rainfall readings (± SE) from the Sundays River Valley irrigation ponds, Eastern Cape of South Africa. Sourced from the South African Weather Service (SAWS) station in Addo Elephant National Park.

Water quality parameters of temperature and, conductivity were measured seasonally (Table 2.1). Temperature and conductivity were the parameters with considerable variation, ranging from 12 °C to 28 °C. Conductivity ranged around 430 μs/m and pH was slightly alkaline at 9.2.

Table 2.1: The mean and range for water quality reading taken during the seasonal readings from August 2013 till March 2015 in the Sundays River Valley irrigation ponds, Eastern Cape. pH, electrical conductivity (μs/m), and temperature (°C) were measured using a Hanna HI98129 Combo pH and electrical conductivity meter (HANNA Instruments Inc. Woonsocket, USA).

Parameter	Mean	Range
pH	9.2	8.5 - 10.4
Conductivity	430	316 - 750
Temperature	20.7	12.6 - 28

Survey sites and sampling methods

In total 10 ponds were surveyed (Figure 2.5). These were HBT1 (Habata), STL (Stillerus), SRS6 (Sun River Citrus 6); SRS4 (Sun River Citrus 4); VG (Vegin Sel), DB (Dunbrody); AVO (Avoca); DC (Disco Chicks); MK (Miskruier); RB (River Bend). The width of the ponds ranged 27 – 128 m with a mean of 74 m. The length ranged 59 – 270 m with a mean of 136 m. The minimum depth was 2 m and a maximum depth of 4 m and a mean depth of 1 m.

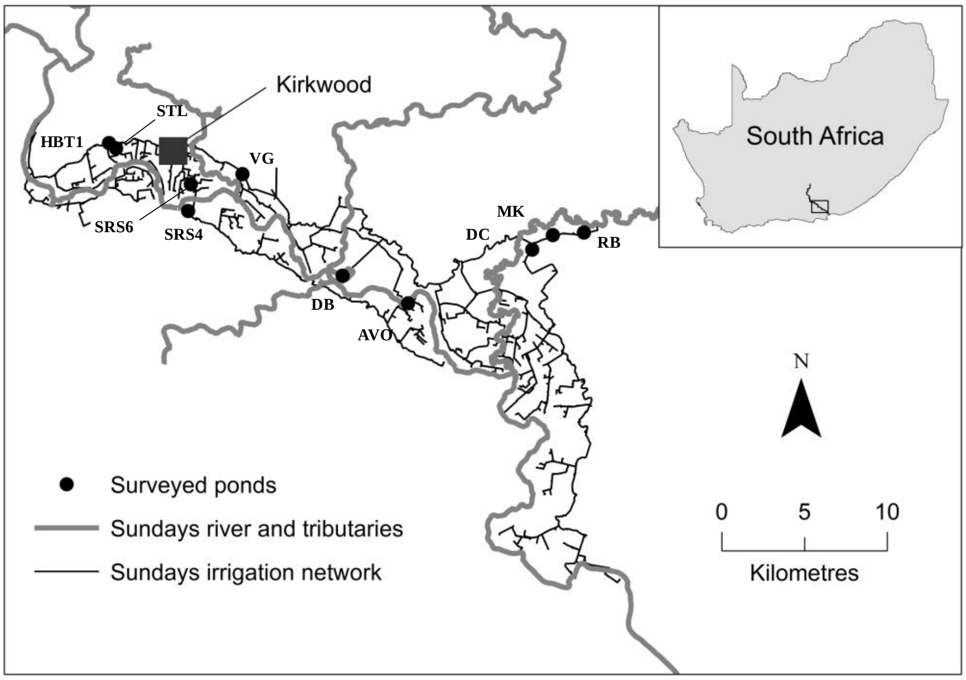

Figure 2.5: Sundays River Valley Irrigation ponds in the Eastern Cape, South Africa, HBT1 (Habata), STL (Stillerus), SRS6 (Sun River Citrus 6); SRS4 (Sun River Citrus 4); VG (Vegin Sel), DB (Dunbrody); AVO (Avoca); DC (Disco Chicks); MK (Miskruier); RB (River Bend).

Fishes were sampled using a 30 m × 2 m seine with 12 mm mesh wings and an 8 mm mesh size cod-end was used. For every seine haul, the net was deployed in a semi-circle near the centre of the pond from an inflatable boat and retrieved into a corner or the centre of a pond wall (see Figure 2.6). Seine hauls, typically 2-3 per pond, were conducted until a maximum of 100 *G. callidus* were collected per sampling event. Once the required *G. callidus* samples were collected the fish were anaesthetised with an overdose of clove oil (1mg L^{-1}), and a subsample was placed in 10% buffered formalin for preservation. These samples were later removed from the formalin and stored in 70% ethanol for analysis. Another subsample of 50 fish was kept on ice for age and growth analysis as formalin destroys otoliths. In the laboratory, biological assessments included the measurements of each sample for total length (TL) to the nearest 0.1 mm using a pair of callipers and the total weights to the nearest 0.01 g and subsequently dissected for biological analysis. Details are provided in the relevant chapters (see Chapter 3 for age and growth, Chapter 4 for reproduction and Chapter 5 for diet).

Figure 2.6: Sampling of the Sunday's River Valley Irrigation pond in the Eastern Cape of South Africa. **A**: inflatable boat is driven out from the beach, with a person feeding out the polypropylene line; **B**: the net is deployed until the bag is reached, then the inflatable is turned parallel to shore and reversed slowly as the net is deployed into the water; **C**: polypropylene ropes are pulled in evenly until the net reaches the beach; **D**: the net is pulled-in evenly, ensuring that that the lead line stays at least in line with the float line. Photos by Tatenda Dalu.

CHAPTER 3

REPRODUCTIVE BIOLOGY OF *GLOSSOGOBIUS CALLIDUS* (TELEOSTEI: GOBIIDAE) FROM IRRIGATION IMPOUNDMENTS IN THE SUNDAYS RIVER VALLEY OF THE EASTERN CAPE, SOUTH AFRICA

INTRODUCTION

Many members of the family Gobiidae exhibit male parental care for the eggs and are iterogenous (repeat) spawners in an extended reproductive season (Corkum *et al*., 1998; Torricelli *et al.,* 1985). The speleophilic (hole-nesting) reproductive behaviour of *G. callidus* was described by Wasserman *et al*., (2015). This is similar to the relatively well studied Ponto-Caspian species *Neogobius fluviatilis* (Konečná and Jurajda, 2009), *Neogobius gymnotrachelus* (Grabowska, 2005), *Neogobius kessleri* (Kovac and Coop, 2009), *Neogobius melanostomus* (Corkum *et al*., 2004) and *Proterorhinus semilunaris* (Valová *et al*., 2015) in which males build a nest on any overhead surface such as logs, stones or other hard substrate with one opening and they will guard the nest (Corkum *et al*., 2004). During guarding the males will fan the eggs, with beats of the pectoral fins and sinusoidal movements of the body, whereby they provide nutrients to the eggs, and this behaviour will gradually increase with the increase in time spent in the nest (Torricelli *et al*., 1985).

Koutrakis and Tsikliras (2009) demonstrated that the estuarine-adapted *Pomatoschistus marmoratus* exhibited bi-maturism where males matured later than females. Kovac and Coop (2009) in a study on the life-history of *N. kessleri* demonstrated that this species will change their life-history style from relatively more altricial (least specialised) to more precocial (specialised) depending on the environment. In invasive populations for example, *N. kessleri* reached maturity at a much smaller size than in its native range, absolute fecundity was higher and spawning was at a higher frequency in contrast to the single batch spawning of *N. kessleri* in their native range (Kovac and Coop, 2009). Using the Winemiller and Rose (1992) continuum, the Ponto-Caspian gobies typically group under the opportunistic category when their parental care is considered, but their high fecundity, early maturity, short life-span and parental care can be grouped under the equilibrium category.

There have been a few studies on the native *G. callidus*, but the research conducted shows that the species is a rapid and successful colonizer of novel habitats such as irrigation ponds (small farm dams) in the Sundays River valley (Woodford *et al*., 2013). It is therefore likely that this species will have similar reproductive traits as invasive Ponto-Caspian gobies. The purpose of this chapter was to provide data on reproductive traits of *G. callidus* that would provide a baseline for future intra-specific research and allow for inter-specific comparisons with other goby species. Specifically, this chapter investigates gonadal development, length at maturity, duration of the spawning season and the spawning frequency.

Gonadal development

According to West (1990), the identification, description and understanding of gonadal developmental stages are an essential element in reproductive studies, because they are essential for defining fecundity, size or age at first maturity, and spawning season (West, 1990). However, precise determination of gonadal development and maturity status depends on correct estimation of gonadal development (Núnez and Duponchelle, 2009). The methods used for the evaluation of ovary development stages in fish include macroscopic staging (i.e. the external appearance of the gonads) and histological assessment. Macroscopic methods are fast and inexpensive but, unfortunately, lack the accuracy of histological methods (West, 1990). Histological analyses are more time consuming and expensive, but yield the more accurate information and provide less ambiguity in assigning maturity status (Abascal and Medina, 2005; Midway and Scharf, 2012; West, 1990; Zeyl *et al.*, 2013), provides precise details on the fish`s reproductive cycle (Abascal and Medina, 2005). Thus reproductive assessments often use a combination of macroscopic and histological methods because histological validation of macroscopic observation is essential because the use of macroscopic staging alone has been shown to overestimate the length and age at maturity, which may bias the interpretation of population dynamics (Booth and Weyl, 2000; West, 1990). For this reason, the current thesis uses histology to validate macroscopic staging.

Maturity and spawning period

Accurate understanding of maturity is a key element in ecology as this provides information on the reproductive potential of fish populations (Williams, 2007). The size at maturity plays an important role as it is a trade-off between producing successfully at a larger size and the risk of mortality before reproducing (Helfman *et al*., 2009). The determination of maturity using length at first maturity, 50% and 100% is essential to understand the fish spawning potential (Winemiller and Rose, 1992). As the proportion of fish approach maturity, their gonads increase in volume (and weight) which peak during the height of the spawning season. As a result, spawning period is traditionally determined by monitoring periodic changes in gonado-somatic index (GSI) and the proportion of ripe and spent fish (Zeyl *et al*., 2013). Traditionally, the GSI is combined with visual assessment because the appearance of spent fish can provide a more detailed indicator as to when the spawning season begins and ends using histological indicators such as the presence of post ovulatory follicles in the ovaries (Hunter and Golberg, 1980).

Fecundity

Fecundity is an important parameter because it provides a quantitative estimate of the reproductive potential (Murua *et al*., 2003). Different species under different environmental conditions will exhibit different fecundity; these are influenced mostly by the size and condition of the fish (Murua *et al*., 2003). Elgar (1990) suggested that there is a tendency of species to produce few large or many small eggs in their reproductive effort. This is an important parameter in describing the life history and fecundity is an essential component in the description of life history traits and for fitting species into reproductive frameworks such as the Winemiller and Rose continuum (Balon, 1975a; Winemiller, 2015; Winemiller and Rose, 1992).

Study objectives and hypotheses

Glossogobius callidus has recently been shown to be a nest guarder (Wasserman *et al*., 2015), and have small to medium body size (Skelton, 2001), a trait associated with both equilibrium and opportunistic strategies in the Winemiller and Rose (1992) continuum. Typically, fishes employing these strategies display high fecundity, moderate maturity short life-span, offer parental care and using this information indicate that *G. callidus* should have traits that are associated with both equilibrium and opportunistic life-history strategy. This will be explored by testing the hypothesis that *G. callidus* display traits in fecundity and reproductive frequency typical of opportunistic strategies but reproduces at a relatively large size to be competitive during guarding.

MATERIALS AND METHODS

Glossogobius callidus was sampled monthly from August 2013 to March 2015 from the Sundays River Valley irrigation ponds in the Eastern Cape Province of South Africa (see Chapter 2 for details).

Laboratory analysis

In the laboratory, all fish were measured for total length TL to the nearest 0.1 mm using a pair of callipers and for total weight to the nearest 0.01g, sexed based on the shape of the urogenital papilla (short and thin for males; long and wide for females) and subsequently dissected. Gonads were visually assessed under a dissecting microscope to confirm the sex determined from the shape of the urogenital papillae (MacInnis and Corkum, 2000b) and then categorised into five macroscopic stages that were developed using standard criteria (Booth and Weyl, 2000; West, 1990) (Table 3.1). The gonads were subsequently removed and weighed to the nearest 0.0001g and the eviscerated body mass of the fish was recorded. Fifty preserved gonadal tissue per sex (i.e. 10 in each of the identified macroscopic stages), were stored in 10% buffered formalin and then later transferred to 70% ethanol for examination.

Table 3.1: Macroscopic staging criteria and expected histological appearance of preserved female and male gonads of *Glossogobius callidus* from the Sundays River Valley Irrigation ponds, South Africa (After Booth and Weyl, 2000).

Stage	**Macroscopic appearance**	**Histological appearance**
Juvenile	Testes and ovaries are strap-like, translucent and almost colourless. Sex cannot be determined	Oogonia, chromatin-nucleolus and pre- and early perinuclear oocytes dispensed in empty lumen. Predominantly spermatogonia are dispersed in partly empty lumen
Resting	Ovaries are fuller and more rounded their walls thick and opaque. Oocytes are not visible. Testes distinguishable as thin band.	Oogonia, chromatin-nucleolus, pre-, early and late perinuclear oocytes increasing in number and size. Lumen still partly empty spermatogonia, spermatocytes are also present.
Developing	Eggs appear white. Larger eggs clearly discernable and opaque in colour. Ovaries occupy about two-thirds of the abdominal cavity. Testes are a pale rose colour and occupy two-thirds of the abdominal cavity.	Ovary contains primary and secondary yolk vesicle oocytes. Testes contain cells in various stages of spermatogenesis, including spermatogonia, spermatocytes, and spermatids and in some cases, small amounts of spermatozoa.
Ripe	Eggs are translucent, round and appear yellow in colour. Ovaries fill the abdominal cavity. Testes are white-yellow in colour and fill the whole abdominal cavity.	Ovary dominated by secondary and tertiary yolk vesicle oocyte. Lobules in testes filled with spermatozoa
Spent	Ovaries have the appearance of deflated sacks, sometimes containing a few remaining eggs. Testes appear as deflated sacks	Post-ovulatory follicles and atretic oocytes visible. Residual spermatozoa and increasing numbers of spermatogonia are present in testes.

Sex ratio

Sex ratio was based on fish larger than the size at first maturity collected during the spawning season (September to December). This was then tested for unity using chi-square test.

Length-weight relationship

The relationships between TL and W were determined by fitting the data to the equation; $W = aL^b$ (Ricker, 1975), where L is the total length (mm), W is the total weight (0.1g), a and b are constants. Regression analysis was employed on data for males and females separately.

Histological Validation

For histological validation, preserved tissues were sent to the University of Pretoria, Section of Pathology, Department of Paraclinical Science, and Faculty of Veterinary Science for histological preparation. Tissues were embedded in paraplast using routine methods, sectioned between 3 and 7µm and stained with Gill`s haematoxylin and eosin. The resulting slides I interpreted for sexual maturity under a Zeiss electron microscopy (SEM) at the department of

Zoology in Rhodes University at variable magnifications, using an adapted version of the gonadal reproductive stage determination index (Table 3.1).

For each developmental stage, a total of 30 oocytes per development stage were measured for cell diameter, -nucleus diameter and number of nuclei per nucleus using imaging software (Sigma-Scan, Jandel Scientific) at varying magnifications of X19, X20, X40 and X100 and the mean and standard deviation were determined. All measurements quoted represent an average of two measurements per cell across the nucleus and perpendicular to each other. Measurements were not corrected for shrinkage attributed to histological preparations (Sivakumaran *et al*., 2003). Microscopic stages of gonadal development were determined based on nuclear and cytoplasmic characteristics of the oocyte or sperm cells. Ovaries and testes were assigned stages based on the most advanced type of oocyte present (West, 1990).

Length at maturity

Before individuals are capable of reproduction, they must reach a certain age or size and their gonadal development (Harold, 2012). Linking length classes to stages of maturity is essential to scrutinise ongoing reproductive processes in the analysed populations as the proportion of ripe fish in a population determines the speed at which the population density can grow (Weyl and Booth, 1999). The mean length-at-maturity (L_{m}) is usually defined as the length at which 50% of all individuals are mature (King, 1995) and was calculated by fitting a logistic function to the proportion of reproductively-capable fish collected during the spawning season and grouped in 5 mm TL classes (Booth and McKinlay, 2001). As a result of the histological appearance of the gonads, reproductively capable fish were considered those in macroscopic stages developing, ripe, and spent. The logistic function was expressed as

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

where $P(L)$ is the percentage of fish that we found mature at the length TL, Lm_{50} is the length at which 50% of the fish in the length class are mature, and δ the steepness of the ogive (Weyl and Booth, 1999). Curve fitting was performed using Solver routine of Microsoft Excel using a least-squares method (Winker *et al*., 2010a). Length at first maturity was taken as the length of the smallest mature male and female in the sample and 100% maturity was the length at which all individuals in the sample were mature. Maximum likelihood estimates of the parameters were obtained by minimising the binomial negative log-likelihood of the form:

$$-LL = -\sum_{i}[miln\,(\hat{P}i) + (ni - mi)ln(1 - \hat{P}i)]$$

where $\hat{P}i$ is the predicted proportion 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 test were used to test the null hypothesis that Lm_{50} values were equal between sexes. Individuals staged such as “developing”, “ripe” and “spent” were used to calculate the length at maturity.

Spawning season

Spawning season was determined by visual assessment of maturity stage of the gonads and by monthly progression of the gonado-somatic index (GSI). Gonado-somatic index was calculated by expressing the gonad mass as a percentage of the eviscerated mass. Monthly GSI was calculated only from individuals that were larger than L_m to determine the length of the spawning season using the equation:

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

Fecundity

Fecundity (absolute fecundity), which is the total number of mature, yolked, and unovulated eggs of a fish prior to spawning (Indira *et al.*, 2013) were estimated by hand counting the eggs under a stereo microscope. Relative fecundity was estimated by dividing the number of all vitellogenic oocytes by the body mass of the fish.

RESULTS

In total 2054 fishes ranging from 21.1 mm to 144.4 mm TL were examined. The largest female fish measured 137.2 mm TL and the largest male fish measured 144.4 mm TL. The morphometric relationships between length and weight are summarised in Table 3.2.

Table 3.2: Length weight regression of *Glossogobius callidus* from the Sundays River Valley in the Eastern Cape, South Africa.

Relationship	Sex	Equation	R^2
TL:WT	M	$W (0.1g) = 0.1401 TL (mm)^{0.0346}$	0.43
	F	$W (0.1g) = 0.1967 TL (mm)^{0.0305}$	0.39

Macroscopic staging was used to validate whether the papillae and the pelvic fin colour could the used to stage the reproductive stages, table 3.3 shows that the urogenital papillae could be used as a suitable structure for staging the reproductive stage for both females and males. Based on genital papillae, 41%fish were confirmed as females, 50% were confirmed as males and 0.1% was undetermined sex (Table 3.3). The sex ratio did (1.1 males: 1 female) did not differ from unity ($x^2 = 0.027$, $df = 1$, $p = 0.87$) and can thus be considered to be 1:1 (Figure 3.1).

Table 3.3: Comparison of papillae and macroscopic stage of *Glossogobius callidus* from the Sundays River Valley in the Eastern Cape, South Africa

Structure	Male	Female
Macroscopic	1026	852
Papillae	1024	788
Pelvic fin colour	751	48

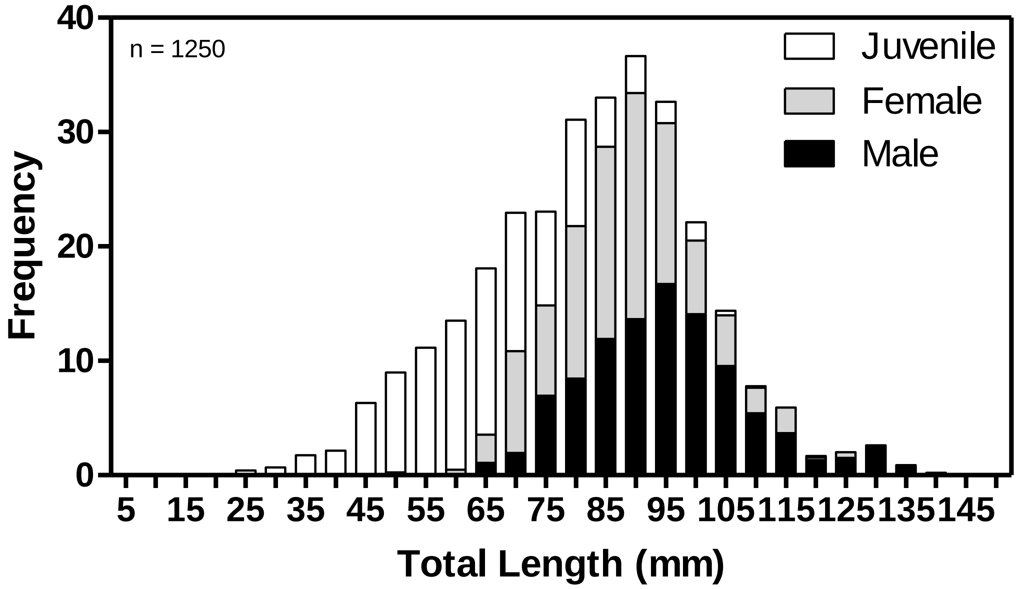

Figure 3.1: Length-frequency distributions for juveniles, males and female species of *Glossogobius callidus* sampled from the Sundays River Valley Irrigations ponds.

Validation of macroscopic staging criteria

There were differences between the macroscopic and histological stages. Seven different gonadal development stages (Oogonia, Chromatin-nucleolus oocyte, Perinuclear oocyte, Primary yolk vesicle, Secondary yolk vesicle and tertiary yolk vesicle, atretic oocytes) were distinguishable based on nuclear and cytoplasmic characteristics. Ovaries within the ‘juvenile’, ‘resting’ and ‘developing’ stages (Figure 3.2A-C) contained oogonia, chromatin-nucleolus and perinuclear oocytes contained numerous nucleoli. The ‘resting’ stages was similar to the ‘developing’ stage with oogonia, chromatin-nuclear oocyte, all perinuclear stages and as traces of primary yolk vesicle oocytes evident (Figure 3.2B-C). In males, there were greater differences between histological and macroscopically assessment of the testes (Figure 3.3A-E). Macroscopic assessment revealed no difference between the “juvenile” and the “resting”; stages they were similar both in size and colour (Figure 3.3A-B). Most attributes that contributed to the different stages were mainly colour and size of the testes with the “developing”, “ripe”, and “spent” stages having more orange and larger size (Figure 3.3C-E). When using histology, the “juvenile” stage showed an empty lumen, whereas, the “resting” stage consisted on spermatids and these increased with each stage (Figure 3.3A-B).

Table 3.4: Mean (± standard deviation) of various stages of oogenesis in *Glossogobius callidus*. Measurements are representative of the fixed material and have not been corrected for shrinkage or swelling attributed to histological preparation. All diameters are an average of two measurements per cell, across the nucleus and perpendicular to one another. N = number of cell measured.

Stage	**Cell diameter (µm)**	**Nucleus Diameter (µm)**	**Number of nucleoli**	**n**
Oogonia	-	-	-	30
Chromatin-nucleolus oocytes	-	-	-	30
Perinuclear oocytes	111 ± 45	54 ± 24	3 – 13	30
a) Pre-	135 ± 24	45 ± 24	4 – 15	30
b) Early-	170 ± 58	78 ± 24	3 – 4	30
c) Late-	261 ± 112	135 ± 48	3 – 4	30
Primary yolk vesicle oocytes	235 ± 97	74 ± 22	3 – 15	30
Secondary yolk vesicle oocytes	264 ± 59	-	-	30
Tertiary yolk vesicle oocytes	299 ± 104	73 ± 28		30
Atretic oocytes	287 ± 91	-	-	30

The coarser and grainy visual appearance of the ovaries in the ‘developing’ stage was due to the appearance of primary vesicle oocyte. The ‘ripe’ stage contained all oocyte stages, being dominated by vitellogenic oocyte with final maturation mainly characterized by the dominance of secondary and tertiary yolk vesicle oocytes similar in size to migratory nuclei (Figure 3.2D). Only ‘spent’ ovaries contained evidence of atresia, with atretic vitellogenic oocyte found throughout the ovary (Figure 3.2E).

Table 3.5: Gonad developmental stages and the equivalent histological and macroscopic characteristics for female Glossogobius callidus from the Sundays River Valley Irrigation ponds.

Stage	**Macroscopic characteristics**	**Histological characteristics**
Juvenile	Gonad appears as thin gelatinous band and hard to distinguish	Oogonia small compared to nucleus in a lightly 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 radiate 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;

Histological observation were generally consistent with macroscopic staging but there were oocytes that contained more than one stage therefore ‘developing’, ‘ripe’ and ‘spent’ stages should be grouped together as they are capable of spawning and should be used for determining maturity (Table 3.5).

The first stage oogonia main characteristics were their small size compared to a relatively large nucleus to cytoplasm in a lightly basophilic cytoplasm (Figure 3.2A). The chromatin-nucleolus oocyte stages were larger than oogonia, mainly characterised by the large centrally located nucleus compared to the cells size, within a lightly basophilic cytoplasm (Figure 3.2B)

Primary yolk vesicle oocytes stage had a uniformly basophilic cytoplasm but the end of this stage was marked by the formation of the zona radiata (Figure 3.2C). Perinuclear oocyte increased in size, containing numerous nucleoli and the oocytes were strongly basophilic (Table 3.4). Pre-perinuclear oocytes were polygonal in shape, with the nucleus containing multiple nucleoli (Table 3.4). The early perinuclear oocyte were slightly less polygonal in shape and contained three or four very distinct nuclear and a couple of less smaller ones and late perinuclear oocyte increased in size and were more ovoid in shape, containing primarily nucleoli that were well arranged in the nuclear wall. The formation of the zona radiata and zona granulose occurs in the late-perinuclear oocytes (Figure 3.2C).

The secondary yolk vesicle oocyte stage was initiated by the acidophilic (red-staining) yolk globules. The ovaries become increasingly filled during recrudescence so that at this stage hardly any empty lumen is left (Figure 3.2D).

The tertiary yolk vesicle oocyte stage was characterized by the presence of atretic oocytes. Phagocytes invade and accumulate around leftover oocytes to initiate their dissolving process, leaving empty lumen in the ovaries. All other oocyte stages are still present in spent ovaries or begin to develop again (Figure 3.2E).

Atretic oocyte stage was characterised by phagocytes invading and accumulating around leftover oocytes to initiate their dissolving process, leaving empty lumen in the ovaries. All other oocyte stages are still present in spent ovaries or begin to develop again (Figure 3.2F).

Table 3.6: Gonad developmental stages and the equivalent histological and macroscopic characteristics for male *Glossogobius callidus* from the Sundays River Valley Irrigation ponds.

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 speramtozoa

In males, there were greater differences between histological and macroscopically assessment of the testes (Figure 3.3A-E) (Table 3.6). Macroscopic assessment revealed no difference between the “juvenile” and the “resting”, they were similar both in size and colour (Figure 3.3A-B). Most attributes that contributed to the different stages were though mainly colour and size of the testes with the “developing”, “ripe” and “spent” stages having more orange and larger size (Figure 3.3C-E). When using histology the “juvenile” stage showed an empty lumen, whereas the “resting” stage consisted on spermatids and these increased with each stage (Figure 3.3A-B).

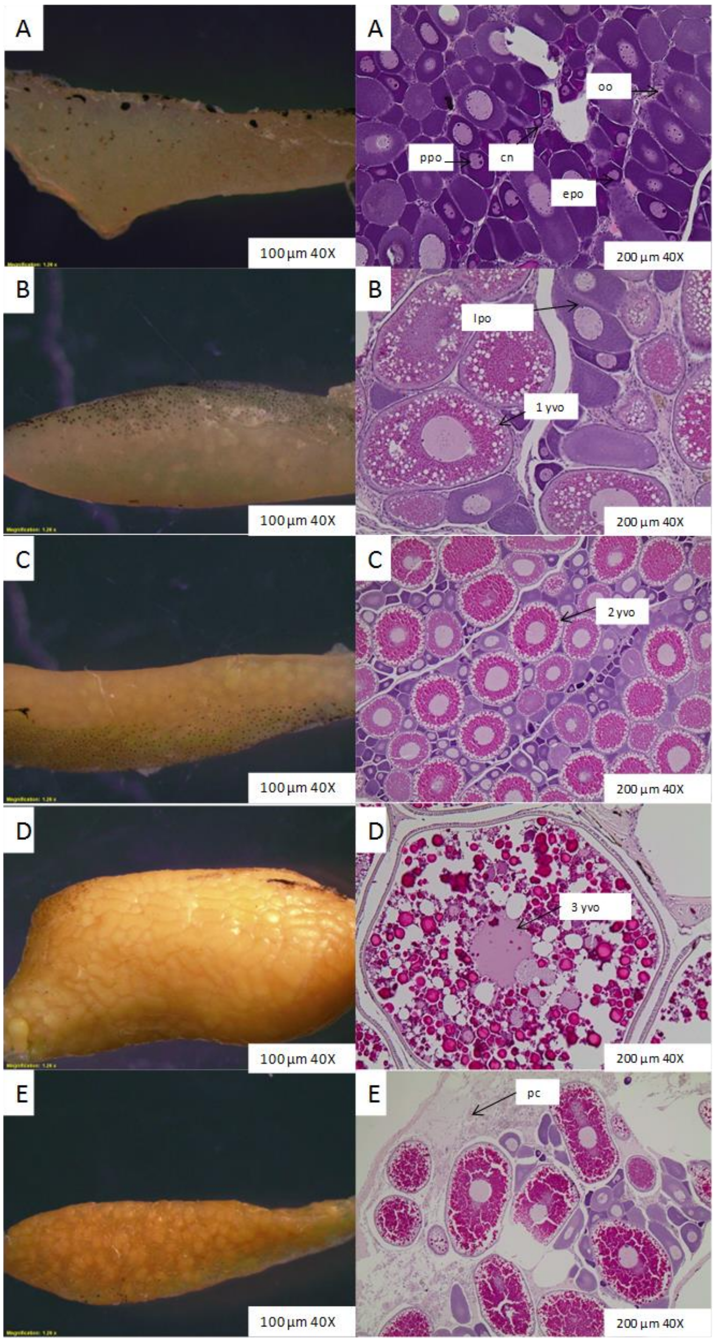

Figure 3.2: Whole and transverse-sectioned ovaries of *Glossogobius callidus* from the Sundays River Valley irrigation ponds that are macroscopically and histologically classified as: A: 'juvenile', B: 'resting', C: 'developing', D: 'ripe', E: 'spent'. **A**, 'juvenile' ovary containing oogonia (oo), chromatin-nucleolus oocyte (cn), perinuclear oocytes, pre- (ppo), early- (epo) perinuclear oocytes. **B**, 'resting' ovary containing (lpo) late perinuclear oocyte and primary yolk vesicle oocyte with cortical alveoli forming in the periphery of the cytoplasm marks the onset of maturation. **C**, 'developing' secondary yolk vesicle appears with the sequestration of vitellogenic yolk, with the yolk globules. **D**, 'ripe' tertiary yolk vesicle oocytes are characterized by cortical alveoli migrating to the cytoplasm and the cytoplasm dominated by yolk globules. **E**, 'spent' atretic oocytes characterised mainly by phagocytosis by invading phagocytes. All sections stained using haematoxylin and eosin. Magnification was at 20X, 10X and 40X.

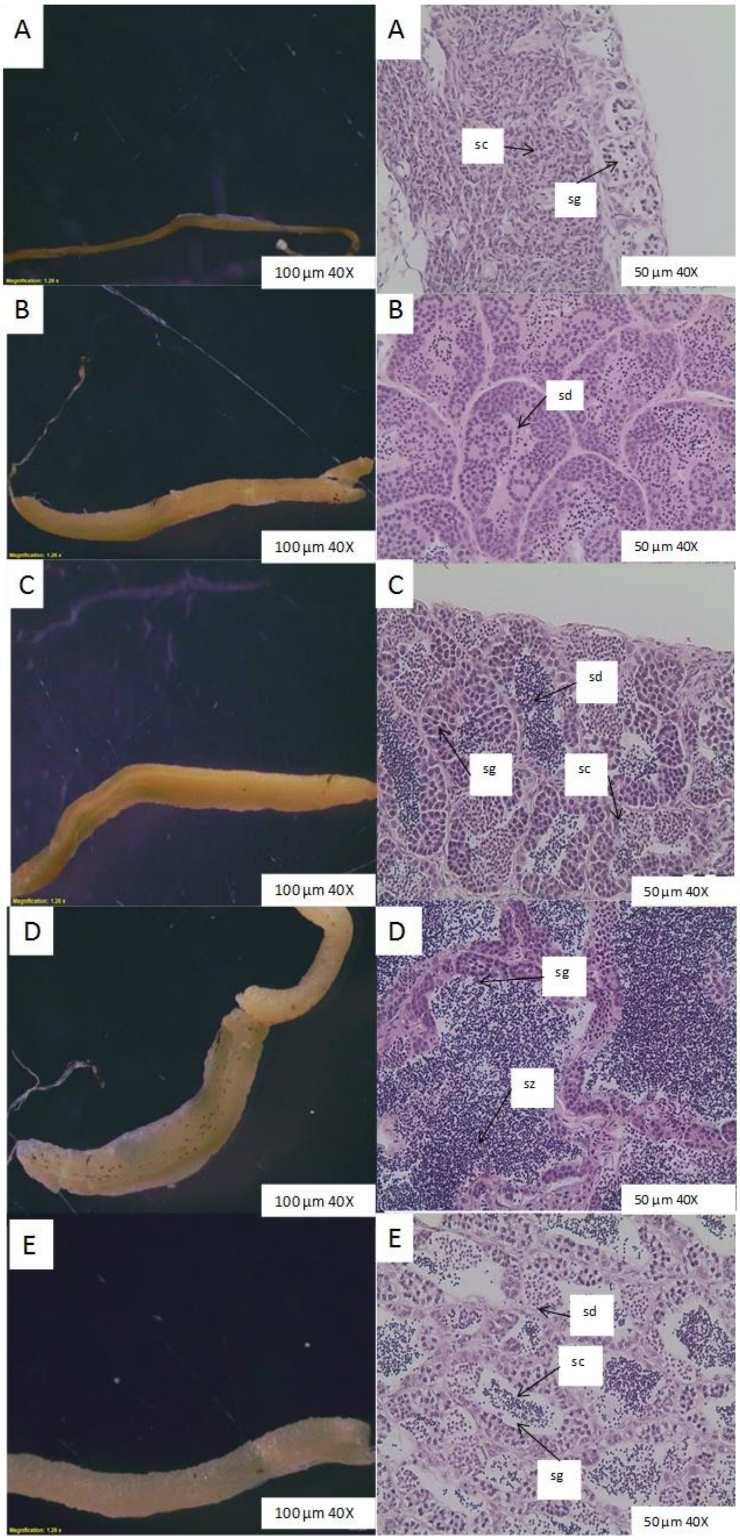

Figure 3.3: Whole and transverse-sectioned testes of *Glossogobius callidus* from the Sundays River Valley irrigation ponds that are macroscopically and histologically classified as: **A**: 'juvenile', **B**: 'resting', **C**: 'developing', **D**: 'ripe', **E**: 'spent'. Sg = spermatogonia; sc = spermatocycte; sd = spermatids; sz = spermatozoa. Magnification was at 20X.

Length at maturity (L_m)

The smallest mature male sampled was 60 mm TL and the smallest mature female was 65 mm TL. All males larger than 60 mm TL and all females larger than 65 mm TL were mature. The logistic function fitted to the proportion of mature individuals in each length classes are shown in Figure 3.4. For females, L_m was 70 mm TL and, for males, L_m was 72 mm TL. There were no significant differences between sexes (Likelihood ratio test; $P > 0.05$).

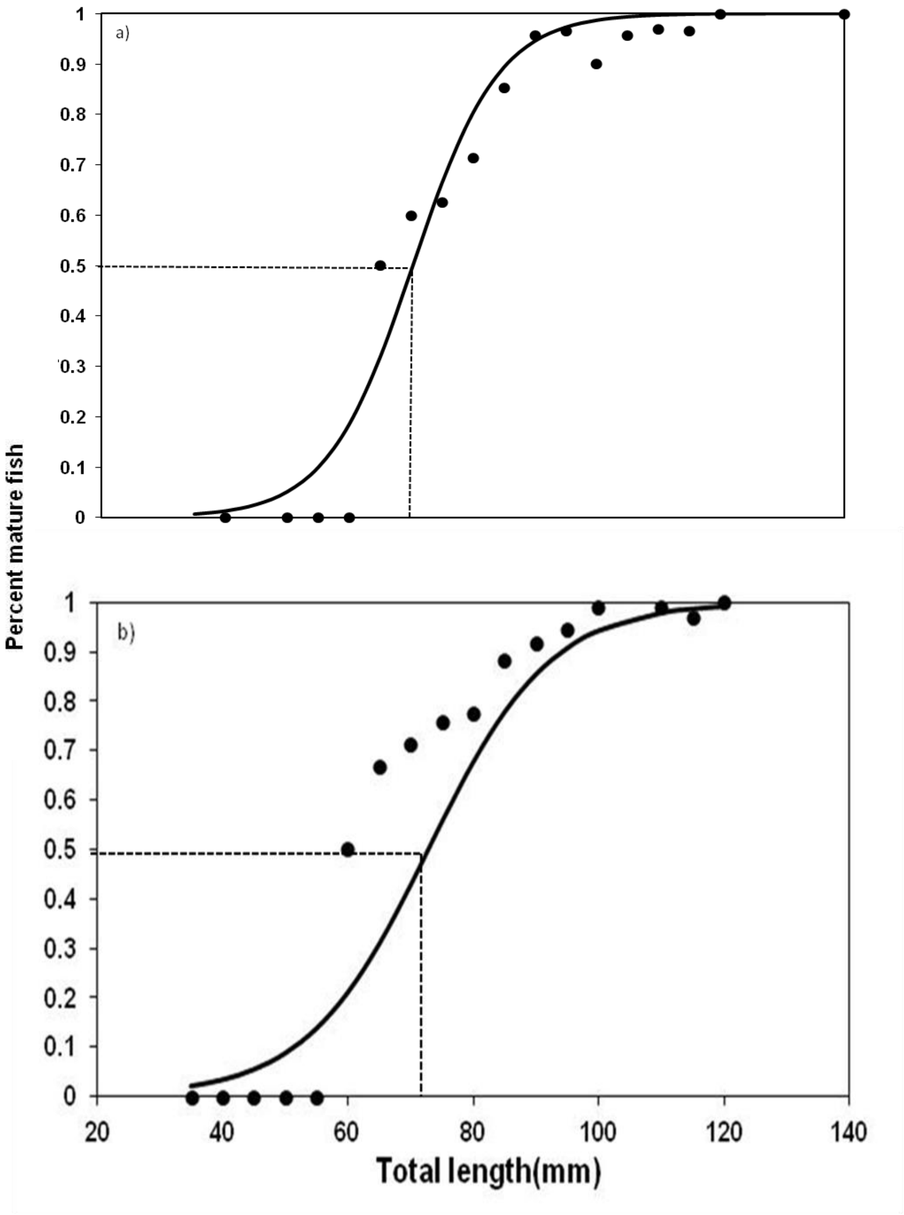

Figure 3.4: Total length (TL) at 50% sexual maturity for *Glossogobius callidus* (a) females and (b) males from the Sundays River Valley Irrigation ponds. 128 females and 233 males were used.

Spawning season

A total of 2054 fishes were inspected visually to determine their stage gonadal development. In *G. callidus* ovaries are paired organs, sac-like organs situated in the peritoneal cavity and suspended within the mesovaria. Their size, colour and appearance were consistent with those described in Table 3.1. Gonad development showed a distinct seasonal pattern with the proportion of ripe fish increasing from a low of 0% in winter (June - August) to a peak of 55.7% in summer (December - February) (Figure 3.5). This pattern was repeated for two consecutive spring/summer cycles (Figure 3.5).

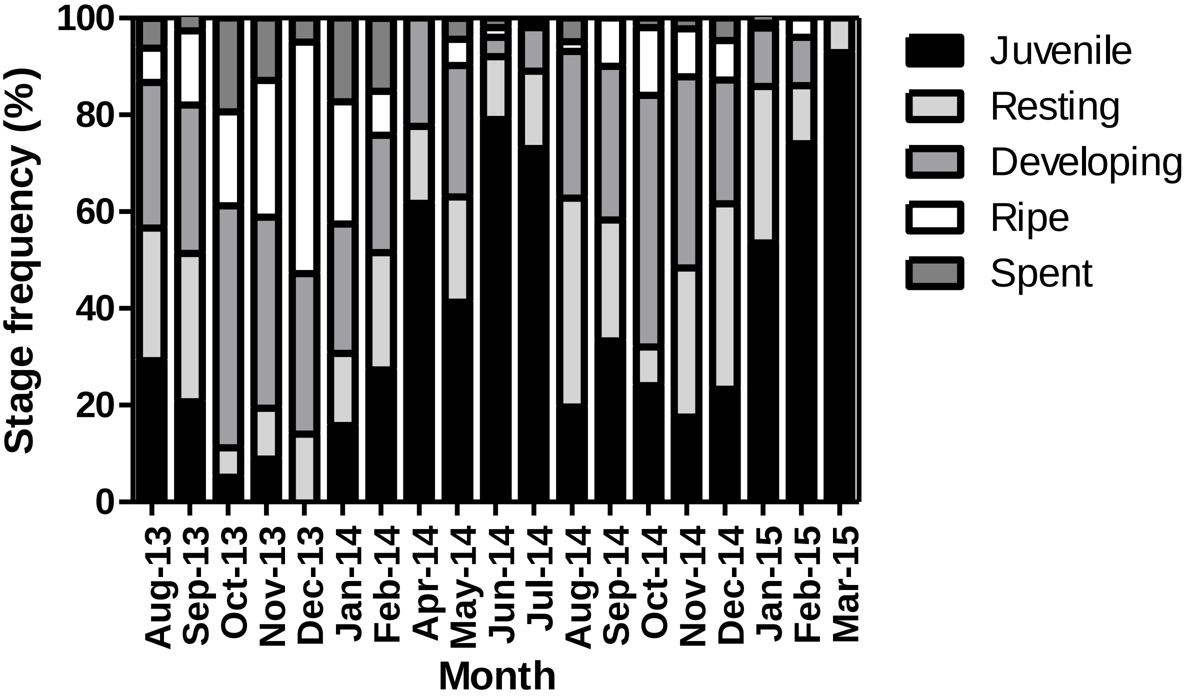

Figure 3.5: Visual assessment of the state of maturity of female gonads of *Glossogobius callidus* samples from the Sundays River Valley Irrigation ponds. Fish were sampled monthly from August 2013 till March 2015.

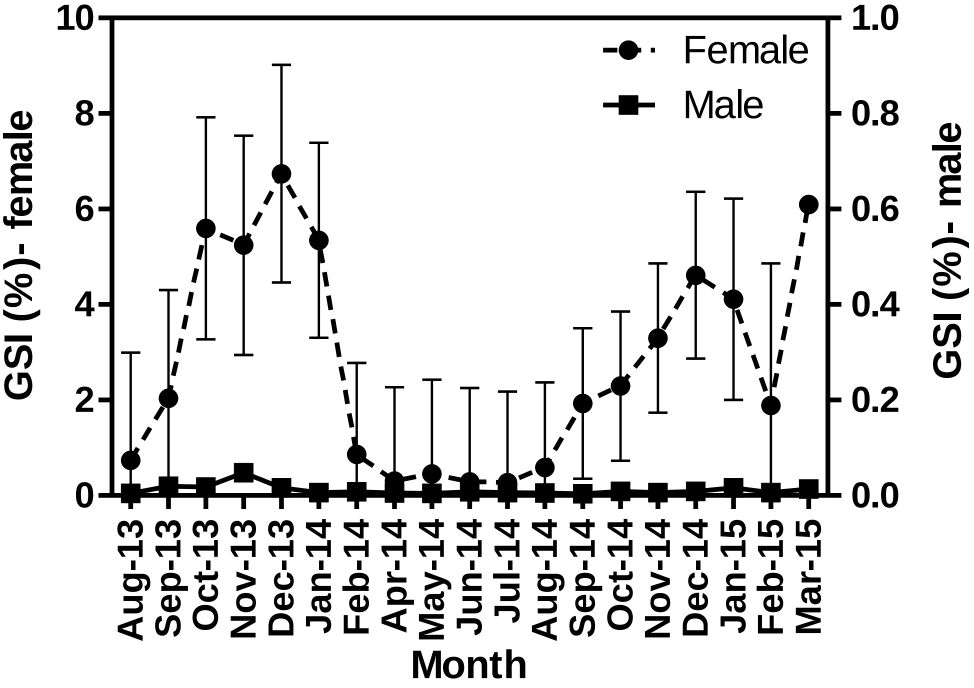

Figure 3.6: Mean (± standard deviation) gonado-somatic index (GSI) for male and female *Glossogobius callidus* sampled from the Irrigation ponds located in the Sundays River Valley, Eastern Cape between August 2013 and March 2013.

GSI followed similar trends increasing from a low late winter of 0.01% (August) and reaching a peak of 0.84% in mid-spring (October) and 0.96% early summer (December), thereafter, it then decreases from January to a low of 0.01% and remains relatively low, until it slowly increases again from 0.01% to 0.03% in mid-winter (Figure 3.6) for females. Males also followed a similar trend from a low late winter of 0.04% and reaching a peak in mid-spring of 0.19% and reaches a peak of 0.47% in early summer (November) and continues to decrease until increasing again in mid-spring and mid-summer.

Fecundity

The number of ripe eggs prior to spawning is defined as fecundity and measures the reproductive potential of a particular fish species. The number of vitellogenic oocytes per fish ranged from 100 to 1000 oocytes per fish. Mean ± S.D absolute fecundity was estimated at 1028.2 ± 131.7 ova/fish. Relative fecundity (number of vitellogenic oocytes per gram of eviscerated fish mass) was estimated at 50 ± 18 ova/fish gram. Fish size and fecundity was positively correlated (Figure 3.7) and the largest oocyte diameter measured was 299 μm.

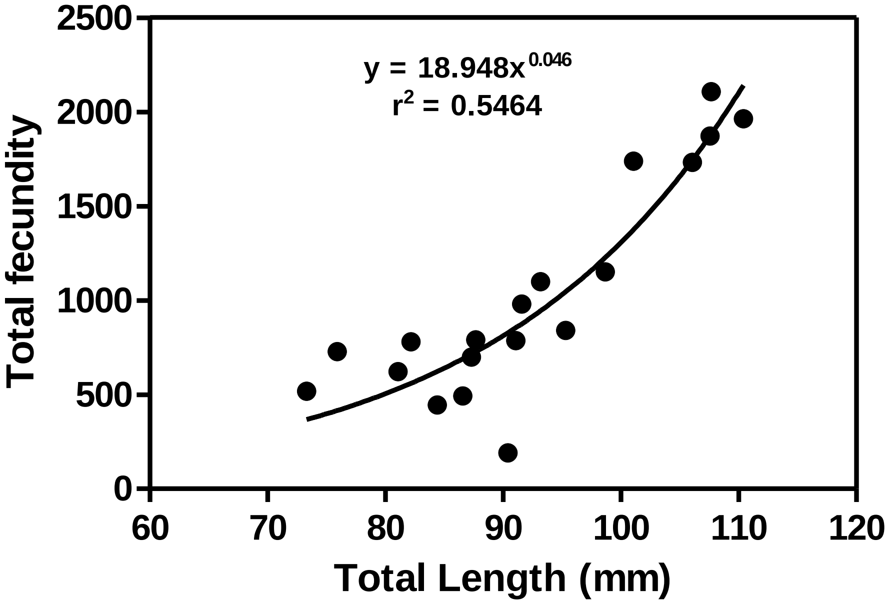

Figure 3.7: Fecundity of *Glossogobius callidus* sampled from ponds located in the Sundays River Valley Irrigation ponds, Eastern Cape between August 2013 and March 2013.

DISCUSSION

In the present study, histological observations showed that some ovaries contained oocytes in perinuclear stage as well as in primary and secondary yolk vesicle oocytes. These histological observations demonstrate that females are capable of repeated spawning in a single season (Weyl and Hecht, 1998). When this information is coupled with the large difference in gonadal investment (based on GSI) between males and females and the behavioural observations on spawning (Wasserman *et al.*, 2015), the data suggest that *G. callidus* is an asynchronous spawner. The different developmental stages of ova found in the ovary suggest multiple spawning and it is likely that a single female spawns several times during the spawning season. Seasonal variation in gonadal development and GSI bimonthly changes indicates a broad spawning season, ranging from mid-spring to mid-summer. This behaviour is common in fishes where males defend territories (Beamish *et al.*, 2005). These findings of a repeated spawning period were consistent with the findings on invasive gobies such as Monkey Goby *N. fluviatilis* (MacInnis and Corkum, 2000b, Valova *et al.*, 2015), Round Goby *N. melanostomus* and the Western Tubenose Goby *P. semilunaris.*

Research on reproductive strategies of fishes is necessary to evaluate the reproductive potential of individual fish species (Gupta and Banerjee, 2013). To understand these strategies requires a combination of available assessment methods such as fecundity, size at maturity and gonado-somatic index (GSI), thought to provide insights on the changes of gonadal development (Booth and Weyl, 2000). GSI, as indicator of the spawning period of teleost's, has been considered more appropriate when associated with other indicator of reproduction (DeVlamming *et al*., 1982) because it may be an imprecise indicator for gonadal stage (Murua *et al*., 2003; Sivakumaran *et al*., 2003). The description of the phases of gonadal development is of great importance for understanding the dynamics of the gonad and to assess reproductive mechanisms of a species (Ferreri *et al*., 2009).

The issues regarding macroscopic staging have been previously reviewed by (West, 1990) and are demonstrated in this study. Even though many fishes have been assigned maturity based on visual inspection, there have been quite a number of methods used to validate these outcomes yet histological validation is simply the benchmark in fish reproductive biology (Midway *et al*., 2013). It was also noted by Booth and Weyl (2000) that histological studies are much more reliable and, according to West (1990), should be based on the most advanced oocytes present, regardless of their number. The histological results in this study suggest that the visual macroscopic criteria were adequate to stage the gonadal recrudescence of *G. callidus* that in conjunction with GSI was used to demonstrate that this species had a spawning season that extends from October to February (see Figure 3.6). Although histological staging agreed with the macroscopic staging, there was rather much detail left out, such as the cellular structure and their prevalence during the entire reproductive cycle (Tomkiewicz *et al*., 2003). Macroscopic staging and GSI alone, therefore, are not able to describe the reproductive status and whether these fish are capable of repeated spawning, hence the need for histology.

In addition to comparisons of spawning behaviour, the determination of traits such as maturity and fecundity allows for comparisons between species (see Table 3.7). There was no significant difference between the males and females and they both matured at lengths of between 50 and 60% of their maximum size. These traits together with the estimates of fecundity allowed for comparisons between species (see Table 3.7). In comparison with other gobies *G. callidus* matured similar relative to *G. niger* (Miller, 1986) and *P. minutus* (Claridge *et al*., 1985) and have a similar fecundity to *A. minuta* (Miller, 1986) and *N. melanostomus* (Skora *et al*., 1999).

Table 3.7: Life history comparison of *Glossogobius callidus* and other goby species according to FISHBASE (www.fishbase.org).

Species	**Sex**	**Lmax**	**Lmat**	**Lmax/Lmat**	**Fecundity**	**Spawning**	**Main Ref**
G. callidus	M	146 TL	72	0.5		guarder	This study
	F	115 TL	70	0.6	$F_{A\ 1028}$		This study
Proterorhinus semilunaris		90 SL	64	0.7	-	-	1
Zosterisessor ophiocephalus		250 TL	163	0.65	15,000	guarder: nester	2
Pomatoschistus minutus		110 TL	61	0.55	3,654	guarder: nester	3
Gobius paganellus		130 TL	88	0.67	3,076	guarder: nester	4
Gobius niger		180 TL	105	0.58	2,449	guarder: nester	5
Aphia minuta		79 TL	45	0.56	1,573	guarder:	5
Neogobius melanostomus		246 TL	85	0.34	1,225	guarder: clutch tender	6
Pomatoschistus lozanoi		80 TL	58	0.725	1,003	-	5
Neogobius fluviatilis		200 SL	88	0.44	774	guarder: clutch tender	7
Proterorhinus marmoratus		150 TL	79	1.89	1,386	Guarder: nester	7

1 = Freyhof and Naseka (2007); 2 = Miller (1984); 3 = Claridge *et al*., (1985); 4 = Breder and Rosen (1996); 5 = Miller (1986); 6 = Skora *et al*., (1999); 7 = Lelek (1987)

In conclusion, *G. callidus* matures at a similar size, with no significant differences in length at maturity between males and females, a trait well observed in other gobies. *Glossogobius callidus* has moderate fecundity and provides parental care (Wasserman *et al*., 2015) that together with the extended spawning season and it appears that females maintain ripe ovaries for an extended periods during mid-summer and mid-spring suggests a high reproductive output and explains why this species was able to rapidly colonise irrigation ponds. This high reproductive output together with propagule pressure would mean that *G. callidus* reached higher numerical densities enabling them to successfully colonize irrigation ponds. While fecundity, maturity and spawning frequency are important components of understanding the life-history, these need to be coupled with information of age and growth to allow for the interpretation of these data on the context of rates. To understand the life-history patterns of *G. callidus* and to complete the framework, information on the longevity and the age at maturity as these form the bases for understanding the life-history strategy employed my *G. callidus* in these irrigation ponds and this is the focus of Chapter 4.

CHAPTER 4

AGE AND GROWTH OF *GLOSSOGOBIUS CALLIDUS* (TELEOSTEI: GOBIIDE) FROM THE SUNDAYS RIVER VALLEY IRRIGATION IMPOUNDMENTS, EASTERN CAPE OF SOUTH AFRICA

INTRODUCTION

The estimation of age and growth of fishes is fundamental in understanding their life history and population dynamics (Booth *et al*., 1995; Campana, 2001; Weyl and Booth, 2008). In the context of life histories, the determination of longevity, growth rate and age at maturity are important traits (Winemiller and Rose, 1992). Accurate ageing is vital for determination of growth, age at sexual maturity, and mortality (Taylor and Weyl, 2012; Winker *et al*., 2010a; Winker *et al*., 2010b). The preferred age-determination method is hard part analysis (e.g., Herman *et al*., 2000; İlkyaz *et al*., 2011; Kanyerere *et al*., 2005; Sabah and Khan, 2014; Winker *et al*., 2010b), which involves the counting of periodic growth increments formed in hard tissues in response to cycles in environmental (e.g. temperature) and biological (e.g. spawning) variables that influence metabolic rate (Campana, 2001).

Traditionally, scales have been used for determining ages of teleost fishes because they do not require the sacrifice of fish, are easy to collect and require little preparation for analysis (Niewinski and Ferreri, 1999). Age estimates derived from scales have, however, often proved to be inaccurate and provided unreliable estimates of fish age particularly for older fish because there are difficulties in distinguishing annuli with increasing age (Long and Fisher, 2001; Sabah and Khan, 2014; Taylor and Weyl, 2012). As a result, otoliths are increasingly being used because they grow continuously throughout the life of the fish, and are metabolically inert, and because the annuli are easier to enumerate than circuli in scales (Simmons and Beckman, 2012; Taylor and Weyl, 2012).

Otoliths are calcified structures that are contained in the endolymphatic sac of teleost fishes and are often called ‘ear bones’ (Gauldie and Nelson, 1990; Weyl and Booth, 2008). To enhance the visibility of growth zones, otoliths are often sectioned, polished and/ or stained (Cailliet *et al*., 2001). The verification and precision of readings of the growth-zones is vital to ensure consistency of the interpretation of age and the closeness of the measurements to reality

(Cailliet *et al*., 2006). Accuracy was defined by Panfili *et al*., (2002), as the closeness of the estimate of a quantity to its true value. Spurgeon *et al*., (2015) stressed the importance of age validation to demonstrate that individual fish have been correctly aged. In a review on validation approaches, Campana (2001) listed edge analysis (EA) as the most commonly applied method used to validate the accuracy of age estimation. In southern Africa, EA has been used to validate growth-zone deposition rate for native cyprinid fish's such as the Largemouth Yellowfish *Labeobarbus kimberleyensis*, Smallmouth Yellowfish *Labeobarbus aeneus*, Orange River Mudfish *Labeo capensis* (Ellender *et al*., 2012; Taylor and Weyl, 2013; Winker *et al*., 2010b) and for non-native Common Carp *Cyprinus carpio* (Winker *et al*., 2010a).

While the family Gobiidae is known to the second largest fish family (Thacker, 2009), there is relatively little known of their life-span, growth or mortality rates. This is problematic as understanding of important life-history traits, including the age at maturity and the rate of mortality are dependent on accurate age determination. This knowledge gap, therefore, requires attention. To address this knowledge gap, this study aimed to determine the age and growth of *G. callidus* from the SRIS in the Eastern Cape, South Africa. To do this, the utility of using scales, burnt and un-burnt otoliths for ageing was evaluated Edge Analysis was used to validate the periodicity of growth zone formation on the most appropriate ageing structure, structures were aged and the relationship between age and length determined. Age-at-length data were then used to determine natural mortality rate using both direct (Ricker, 1975) and empirical approaches (Pauly, 1980) to help place the species in a life-history framework. The findings presented here represent the first attempt to provide validated age estimates and the first attempt to determine age-at-length for any *G. callidus* population.

MATERIALS AND METHODS

Samples of *G. callidus* were collected monthly between February 2014 and March 2015, from the Sundays River Valley Irrigation ponds in the Eastern Cape Province of South Africa (see Chapter 2 for details).

Scale preparation and analysis

Scales were removed from under the pectoral fin on the left hand side of the body, from every fish collected from February 2014 to March 2015 (n = 1424); these were cleaned with water and tissue of the rest of the epidermis and preserved between two glass slides. A subsample of scales from 241 fish was read under a dissecting microscope at low magnification (×10). Low magnification was used in order to reduce the bias that will be created by false rings and other artifacts (Taylor and Weyl, 2012). Annuli were only counted if common to two or more scales viewed. Regenerated and not clearly readable scales were excluded from the analysis and the age was determined by counting the number of completely developed annuli (see Figure 4.1A).

Otolith preparation and analysis

To determine the growth rate otoliths were collected. Each fish was measured to the nearest millimetre (TL), sagittal otoliths were removed from all collected fish, these were cleaned, labelled, and stored dry prior to sectioning and interpretation. These otoliths were prepared following methods described by Weyl and Hecht (1999). To enhance visibly of the otoliths rings, one of the otoliths was burned over low intensity ethanol flame until it turned pale brown (Booth *et al*., 1995) (Figure 4.1C). Care was taken not to char the otoliths, as this would obscure the internal structure and margin of the otoliths (Booth *et al*., 1995). All of the otoliths were then set in clear polyester casting resin, sectioned transversely at a thickness of 0.4 mm through the nucleus using a double bladed diamond saw, and then mounted onto microscope slide using DPX microscopy mountant.

Otoliths sections were examined under a binocular microscope using transmitted light at variable magnifications (10 – 40X) and pairs of opaque and hyaline growth zones were counted with no reference to the fish size.

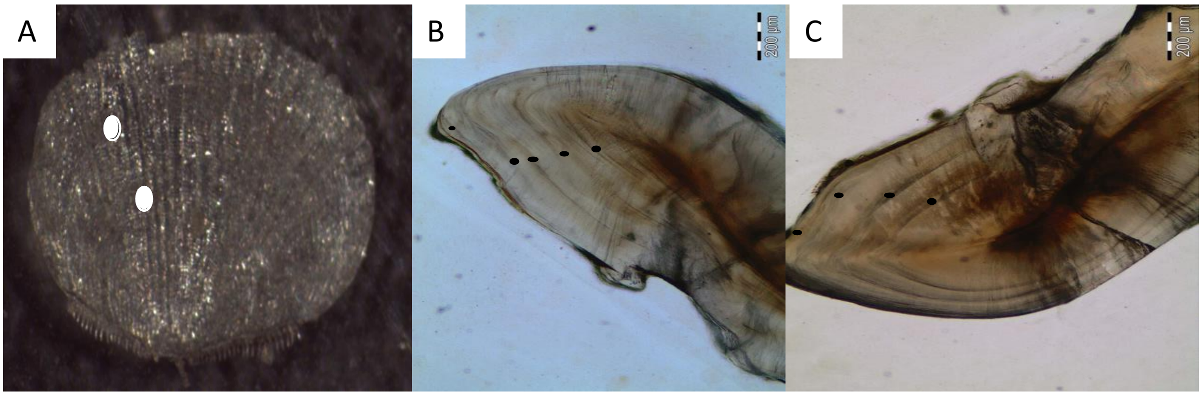

Figure 4.1: Scale and otoliths of *Glossogobius callidus* from the Sundays River Valley Irrigation ponds in the Eastern Cape of South Africa. (A) a scale from a 127 mm TL fish showing 2 annuli; (B) illustrate an un-burnt otolith from a length of 86.1 mm TL fish showing 5 annuli; (C) illustrate a burnt otolith from a 78.1 mm TL fish showing 4 annuli.

Ageing Precision

Relative age estimates were obtained from multiple readings of all structures. Precision between structures was assessed using two readings conducted by the primary reader (Lubabalo Mofu) and results of a subsample reading conducted by a secondary reader (Modiegi Bakane, MSc Candidate, Department of Ichthyology and Fisheries Science). This lead to three readings (two readers) being available to determine relative age estimates for comparisons. The two readings by the primary reader were obtained after the reader gained experience through an initial reading, the results of which were discarded. Readings were separated by a period of at least two weeks to avoid bias through memory of individual structures, and readings were conducted with no reference to biological data for each fish. A relative age estimate was accepted as the mode of the three readings if two of the three readings were the same or the median, if three readings were consecutive. Otherwise the otolith or scale was rejected. Comparisons between structures were, therefore, only possible where age estimates were obtained for both structures, from the same fish, and sample sizes were dependent upon successful readings by the secondary reader. Between-reader comparisons were based on the final age estimate and the relative ages estimated in the three readings.

Precision was assessed according to the proportion of otoliths or scales that were discarded as unreadable during the determination of agreed age estimates (discard rate), agreement of between reader counts, and the average percent error (APE) and coefficient of variation (CV) of growth zone counts calculated from the relative ages assigned by three separate readers. Average percent error (APE) was calculated as described by (Beamish and Fournier, 1981) and the error associated with different relative age estimates was expressed as the Coefficient of Variation (CV) (Chang, 1982).

Average percent error was calculated by the equation:

$$\mathrm{APE} = \frac{1}{N}\sum_{j=1}^{n} \llbracket \left[\frac{1}{\mathrm{R}}\sum_{\mathrm{i}=1}^{\mathrm{R}}\frac{|\mathrm{X}ij-\mathrm{X}j|}{\mathrm{X}j}\rrbracket\right],$$

where N is the number of fish aged, R is the number of times each fish is aged, X_{ij} is the ith age determination of the jth fish, X_j is the average age calculated for the jth fish. This was then multiplied by 100 to become the index of average percent error.

coefficient of variation (CV) was calculated as:

$$\mathrm{CV}j = 100 * \frac{\sqrt{\sum_{i=1}^{R}\frac{(xij-xj)^2}{R-1}}}{Xj},$$

where CVj is the age precision estimate for the jth fish. The CV was averaged across all fish to produce a mean CV.

Validation

The growth zone deposition rate on otoliths was validated using edge analysis. For (EA) the optical appearance of the edge of the otoliths was assessed (under 10 – 40× magnification), and then categorised as opaque zone present or absent. According to Campana (2001), edge analysis is based primarily on the assumption that the frequency of edge zones follows a yearly sinusoidal cycle when plotted against time. The results were noted as Bernoulli variables (1 = present, 0 = absent). The binary results were expressed as the proportion of otoliths with an opaque zone present per month and modelled using a periodic logistic regression (Winker *et al.*, 2010a) expressed in the following form:

$$Logit\left(\hat{\mathrm{O}}\right) = \beta 0 + \beta 1 \sin 2\pi\left(\frac{MOYi}{PE}\right) + \beta 2 \cos\left(2\pi\left(\frac{MOYi}{PE}\right)\mathrm{i}\right),$$

where *logit* is the link function for the binomial distribution, $\hat{O}_i$ is the expected proportion of otoliths with an opaque zone present at the margin for each angular transformed month of any year *MOYi*, *PE* is the assumed monthly periodicity of growth deposition and β_0, β_1, β_2 the regression coefficients (Beamish *et al.*, 2005). Regression parameters were estimated by minimizing the binomial negative log-likelihood function of the form:

$$-LL = -\sum_{i=0}^{11}[\text{mi In}\left(\hat{0}\right) + (\text{n} - \text{m})\text{In}\left(1 - \hat{0}\right)],$$

where n_i is the number of otoliths examined per month and m_i represents the number of otoliths with an opaque zone present on the margin. To test the null hypothesis (H_0) that growth zone deposition is annual, a likelihood ratio test:

$X^2 = 2(LL_{\text{full}} - LL_{\text{reduced}})$,

where LL_{reduced} is the log-likelihood for the reduced model with the constraint that *PE i*s estimated, and *k* is the difference in estimated parameters of the two models. Similarly, the alternative hypothesis (H_a) that growth zone deposition is bimodal where *PE* was fixed at six a significance level of $P \leq$ was used for all test.

Modelling Age

Newly hatched fish were measured during Wasserman *et al*., (2015) spawning experiment. These fish averaged 21.2 mm TL and were included as age 0 fish for modelling age. Preliminary assessments demonstrated highly variable results. As a result, only fish from the Dunbrody Pond were used as this was the oldest pond sampled (35-years) and it was assumed that this particular pond would provide the best representation of the age and growth of the *G. callidus*. The sample of 313 fish collected between April 2014 and January 2015 was split into males (n = 101) and females (n = 112).

As samples were obtained from throughout the year, age estimates were corrected for monthly age using a birth date (January) that corresponded with the height of the breeding season (see Chapter 3) and with opaque zone deposition (see EA Figure 8) as recommended by Weyl and Hecht (1998). For example, a fish sampled in June, with one growth zone visible on its otolith would be assigned an age of 1.5-years. In addition, length measurements for newly hatched fish (Wasserman *et al*., 2015) were used as the length at age 0. Subsequently, the observed length-at-age for male and female *G. callidus* were modelled using the von Bertalanffy growth function, which is described as:

$$L_t = L_\infty (1 - e^{-K\,(t-t0)})$$

where L_t is the length at time *t*, L_∞ is the theoretical asymptotic length, *K* is the Brody growth coefficient and t_0 is the age of a zero-length fish (Ricker, 1975). The model was fitted using a non-linear minimization of the negative log-likelihood of the form

$$-\,\text{In}\, L = n\, In\, \hat{\sigma} + \frac{n}{2}$$

where $\hat{\sigma}$ is the maximum likelihood estimate of the model standard deviation described as:

$$\hat{\sigma} = \sqrt{\frac{\Sigma(Li - \hat{I}i)2}{n}}$$

where Li, Li and n are the observed and the number of data points, respectively. Variance estimates were calculated using parametric bootstrap resampling with 1000 replicates drawn from a normal distribution (Efron, 1982). Standard errors and confidence intervals were constructed using the percentile method of Buckland (1984). Differences in parameter estimates between sexes and growth rate were assessed with a likelihood ratio test.

Natural mortality

An estimate of mortality rate was determined to understand the dynamics of *G. callidus* populations. The instantaneous rates of annual mortality (Z) was estimated by catch curve analysis (Ricker, 1975) using length frequency distribution which were converted to age frequency distribution by means of a normalised age-length key (Butterworth *et al.*, 1989). The mortality from this dataset was then compared to other populations of the family Gobiidae.

RESULTS

A total of 1424 *G. callidus* were collected ranging from 21.1 mm TL to 144.4 mm TL. The maximum number of growth zones counted was 7 for un-burnt otoliths, 4 for burnt otoliths and 3 for scales. Of the analysed otoliths, 363 (25%) un-burnt otoliths were rejected as unreadable, and 1189 (83%) burnt otoliths were rejected as unreadable, and (96.4%) scales were rejected. These represented the number of structures that were considered unreadable by one of the readers or where one of the three readings used to determine the relative age estimate differed by up to two from the three readings. Estimated APE and CV were similar for the “readable” portion of the sample of un-burnt otoliths (APE= 29%, C.V. = 39.4%), burnt otoliths (APE = 30.4%, C.V = 35.1%) and scales (APE = 35.5%; C.V. = 0.46%). Un-burnt otoliths were, however, chosen as the most suitable structure as they were more readable than the other structures. Fish were assigned older ages (6 to 7) for un-burnt otoliths (5 to 6) for burnt and (3 to 4) for scales. Un-burnt otoliths yielded more precise estimates of ages as indicated by lower average percentage error (APE) (Beamish and Fournier, 1981) and coefficient of variation (CV) (Campana *et al*., 1995).

Validation

The frequency of opaque margins on 643 otoliths collected over a one-year period was best described by a unimodal periodic regression model (x^2 = 1.54, , d.f. = 3, P = 0.21) and not by a bimodal periodic regression model was rejected (x^2 = 4.03, , d.f. = 3, P = 0.004). Periodic regression parameters are provided in Table 4.1. The observed and predicted data revealed that the highest proportion of opaque zone deposition in the otoliths occurred in summer (December) when environmental temperatures were at their highest (Figure 4.2). Edge analysis, demonstrated that the deposition rate of growth zones was annual. Subsequent analyses were based on the direct interpretation that 1 opaque/translucent growth zone pair = 1-year of age.

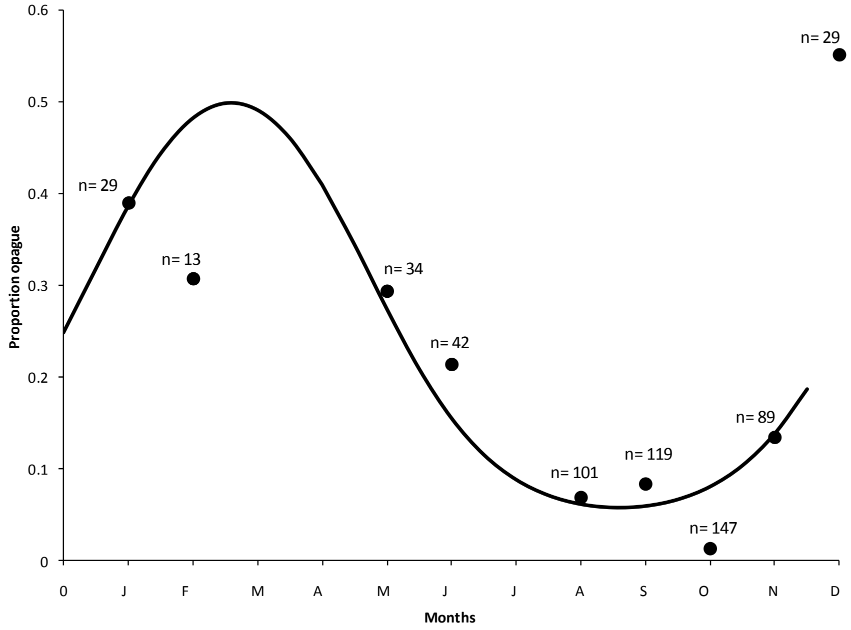

Figure 4.2: Proportion of otoliths with an opaque zone on the margin for *Glossogobius callidus* based on data collected between April 2014 and March 2015 from the Sundays River Valley Irrigation ponds Eastern Cape, South Africa. The predicted unimodal annual cycle of opaque zone deposition using binomial periodic regression, with n representing the number of samples in each month.

Table 4.1: Parameter estimates from the logistic periodic regression analysis predicting the temporal proportion of opaque zone deposition over a one year period for *Glossogobius callidus* in Sundays River Valley Irrigation ponds Eastern Cape, South Africa. The periodicity (PE) was estimated for the full models and fixed for the unimodal and bimodal models.

	Periodic regression models		
Parameter	**Full**	**Annual**	**Biannual**
β_0	-1.42	-1.4	-1.82
β_1	1.49	1.36	0.63
β_2	-0.12	0.29	1.32
PE	11.23	12	6
d.f	4	3	3
ln *L*	222.15	222.84	218.25

Length-at–age

There were no significant differences between sexes (Likelihood ratio test; P > 0.05). Age structure of fish in the sampled ponds is shown in Table 4.2 and age-at-length keys are provided in Tables 4.3, 4.4 and 4.5. The age structure in the ponds differs greatly from the youngest of one year (River Bend) to the oldest of 35-years (Dunbrody), the oldest fish in River bend was 6-years old and this pond was mainly dominated by fish that have reached their asymptote length. The second youngest pond "Disco Chicks", was also dominated by populations that were three years and older and the oldest fish was 7-years old, the oldest known in the species. Ponds older than 5-years had wider age distributions, from fish less than a year old (SRS6 and Dunbrody) to fish as old as 6-years (Table 4.2). Males were found to dominate in length classes of 45 to 100 mm TL females were only found between length classes of 35 to 100 mm TL and were dominate in age group 2 and 3. This then shows that males lived longer than females.

Table 4.2: Comparison of pond age with ages of *Glossogobius callidus* population from the Sundays River Valley irrigations ponds in the Eastern Cape, South Africa.

Pond	**Pond age (years)**	**Age (years)**								
		0	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**n**
Avoca	23			5	31	11	2			49
Dunbrody	35	1	18	31	99	31	16	2		198
Disco chicks	5			16	98	36	20	9	1	180
River Bend	1		2		13	17	13	7		52
SRS4	9	3		11	8	5				27
SRS6	6	9	2	18	22	15	1			67

Table 4.3: Age-length-keys for the entire population of *Glossogobius callidus* sampled from irrigation ponds in the Sundays River Valley, Eastern Cape, South Africa. Age estimated from sectioned sagittal otoliths

Length class (mm TL)	**Age**							
	0	**1**	**2**	**3**	**4**	**5**	**6**	**7**
5	6							
40		1		2				
45		1	2	4				
50		1	1	8	1			
55		4	3	16	4			
60		2	5	26	8	1		
65		2	4	26	5	2		
70			10	22	14	1		1
75		2	11	26	7	3	1	
80		3	8	33	11	5	1	
85			7	33	18	4	5	
90			9	25	17	8	1	
95			9	25	10	7	1	
100			4	8	9	3	2	
105			3	6	3	5	3	
110			1	3	2	4		
115			1	2	3	3		
120			2	2	1		1	
125				1	1	1	1	
130				1	1	3	1	
135			1	1		2		
140				1				
N	6	16	81	238	115	52	17	1

Table 4.4: Age-length-keys for male *Glossogobius callidus* sampled from irrigation ponds in the Sundays River Valley, Eastern Cape, South Africa. Age estimated from sectioned sagittal otoliths

Length class (mm TL)	**Age**							
	0	**1**	**2**	**3**	**4**	**5**	**6**	**7**
5								
35		1						
40		1		2				
45		5	2	4				
50		1	1	8	1			
55		4	3	16	4			
60		3	5	26	8	1		
65		2	4	26	5	2		
70			10	22	14	1		1
75		2	11	26	7	3	1	
80		3	8	33	11	5	1	
85			7	33	18	4	5	
90			9	25	17	8	1	
95			9	25	10	7	1	
100			4	8	9	3	2	
105			3	6	3	5	3	
110			1	3	2	4		
115			1	2	3	3		
120			2	2	1		1	
125				1	1	1	1	
130				1	1	3	1	
135			1	1		2		
140				1				
N		22	81	271	115	52	17	1

Table 4.5: Age-length-keys for female *Glossogobius callidus* sampled from irrigation ponds in the Sundays River Valley, Eastern Cape, South Africa. Age estimated from sectioned sagittal otoliths

Length class (mm TL)	**Age**							
	0	**1**	**2**	**3**	**4**	**5**	**6**	**7**
5								
35		1						
40		1		2				
45		5	1	3				
50		1	1	6	1			
55		3	1	7	2			
60		1	2	8	2	1		
65		2	1	8	1			
70			4	6				
75			3	8				
80			4	6	1			
85			17		2			
90			1	1	1			
95			1	3	1			
100			4	8	9	3	2	
N		14	40	66	21	4	2	

Of the 560 *G. callidus* used to determine the length at age, von Bertalanffy growth parameters and error terms calculated using random error methods (Table 4.6). It was found that the entire population had higher L_∞ and lower K than those of males and females only from Dunbrody. The VBGF growth showed that *G. callidus* has rapid growth where the asymptote is reached in 2-years, relatively the same as maturation, but males reaching the asymptote at a larger size compared to females (Figure 4.3).

Table 4.6: Von Bertalanffy parameter and standard deviation (S.D.) calculated using error methods (Cope and Punt, 2007) for the entire population and for males and females of *Glossogobius callidus* from the Sundays River Valley in the Eastern Cape, South Africa.

	L_∞ (mm)	**S.D.**	**K (year $^{-1}$)**	**S.D.**	**t_0**	**S.D.**
Entire population	91.8	0.09	0.5	0.02	-0.43	0.009
Male	70.3	0.12	1.4	0.01	-0.06	0.003
Female	65.6	0.07	1.8	0.01	-0.05	0.002

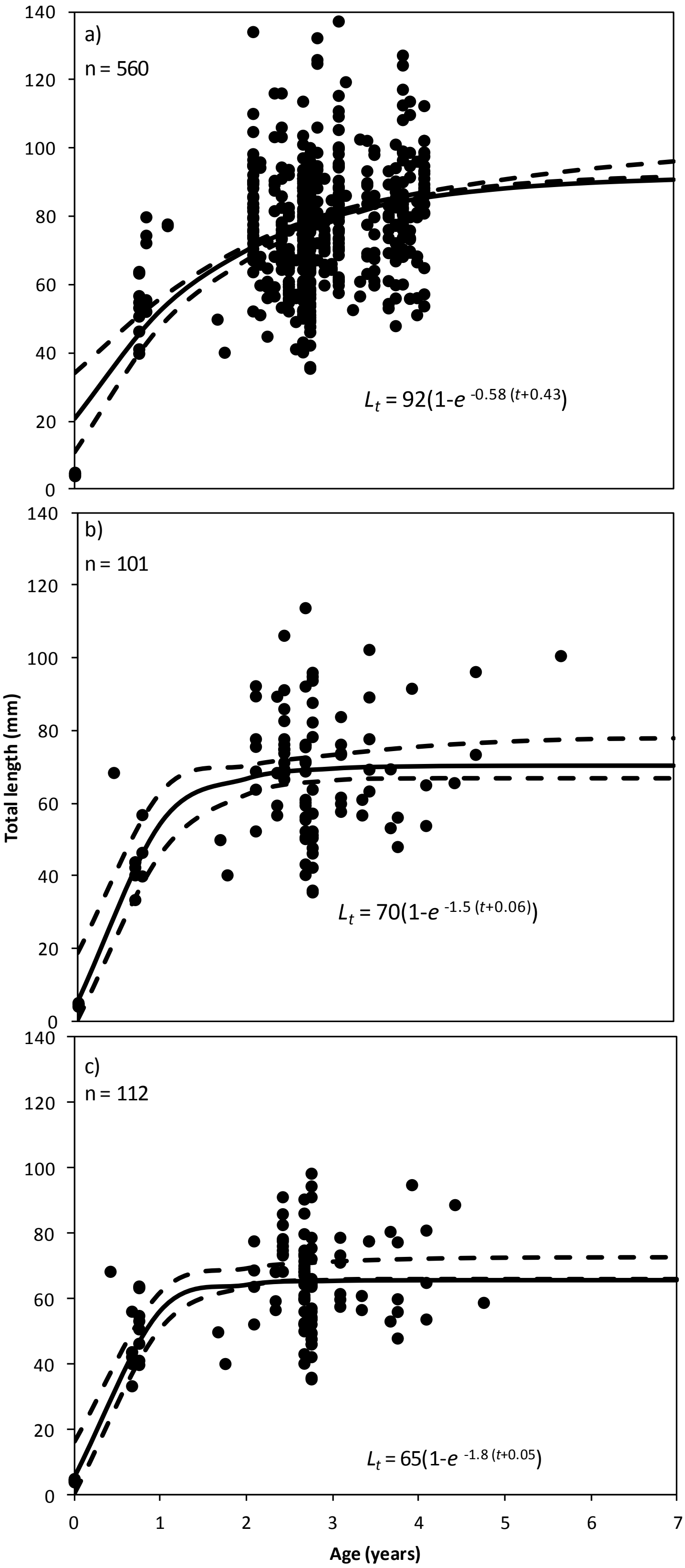

Figure 4.3: von Bertalanffy growth curve for *Glossogobius callidus* from the Sundays River irrigation ponds in the Eastern Cape, South Africa. a) Combined (n = 560); b) male (n = 112); c) females (101).

Mortality

The age-frequency histograms and corresponding linearized catch curves for the entire population (males and females) of *G. callidus* are illustrated in Figure 4.4. The estimated total mortality Z was 1.31 yr^{-1}. As this species is not exploited, Z was taken to equal natural mortality M. (Figure 4.4).

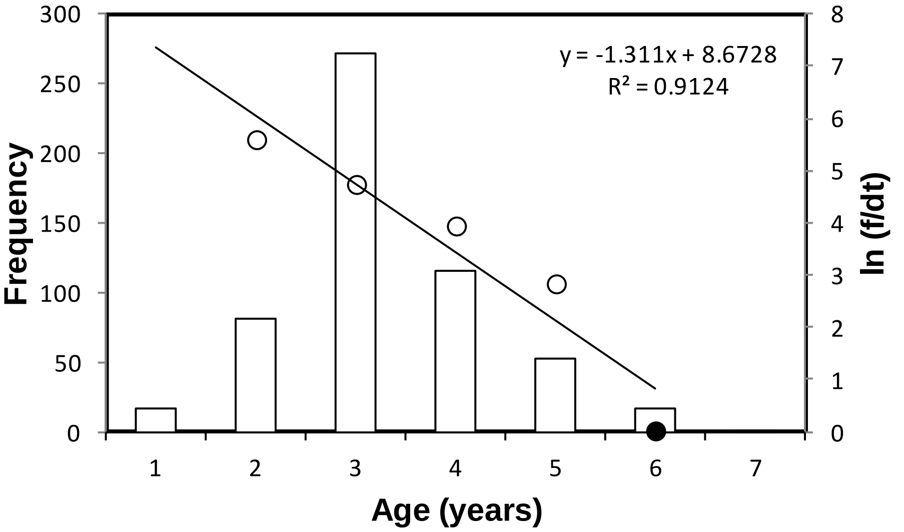

Figure 4.4: Age-frequency histograms and resultant catch curves generated from length frequencies of *Glossogobius callidus* sampled from the Sundays Irrigation ponds in the Eastern Cape, South Africa. The instantaneous rate of mortality (Z) is the slope of the descending limb of the catch curve (closed circles).

DISCUSSION

Glossogobius callidus has a fast growth pattern as the asymptotic length is attained after 2 years, at a similar length to when maturity is attained. This reflects a shift in energy allocation from somatic growth to reproductive energy use, e.g., gonad growth, nesting behaviour, and territorial defence (Weyl and Hecht 1998). For *G. callidus*, the males attained a larger asymptotic length (92 mm TL) compared to females (70 mm TL). This might be because males matured later than females, a trait often documented in this fish family and most likely related to the need for larger size to be competitive when guarding the nest and offering parental care (Kovačić 2006; Sokołowska and Fey 2011).

The determination of age and growth of fishes underpins the basics to understanding life-history (He *et al*., 2008). According to Winemiller and Rose (1992) understanding the life-history traits of fishes is important in determination of population performance. This study successfully aged *G. callidus* using un-burnt otoliths because scales typically had high discard rates, poor readability and resulted in lower estimates of age compared to otoliths. This is not uncommon and has been demonstrated for other teleost species (Long and Fisher, 2001; Taylor and Weyl, 2012). During periods of food limitation and or stress, scales are prone to asymptotic re-absorption (DeVries and Frie, 1996). Scales therefore underestimated the growth of the *G. callidus*; this might be because when *G. callidus* reaches asymptotic length the opaque zones concentrate on the scale margin of older fish. High discard rates for burnt otoliths resulted from burning obscuring internal structures and making the otoliths brittle and unreadable as has been observed in *Neogobius melanostomus* from the middle Danube (Grula *et al*., 2012) and for *Micropterus salmoides* in South Africa (Taylor and Weyl, 2013).

Understanding the manner in which physical, chemical, environmental, and physiological factors are interpreted in fish otoliths, scales and or spines remains one of the fundamentals in understanding fish growth (Winker *et al*., 2010b). The formation of a single opaque band in the otoliths of *G. callidus* during December corresponds with mid-summer, when temperatures are at their highest (Figure 4.2). This may have to do either with the reallocation of resources by fish during the spawning season (Weyl and Hecht, 1998) or because growth zones are often only visible after their deposition and therefore the visual detection lags behind the actual deposition time (Taylor and Weyl, 2013).

In this study, only fish from the 35-year old Dunbrody irrigation pond were used to determine age structure. This was because estimations from an established population were believed to be most suitable for estimating age and longevity and because Woodford *et al*., (2013) demonstrated that population establishment generally occurred within the first decade. Here the oldest *G. callidus* was 7-years old, which is currently the longevity record for this species. It is interesting to note that the age structure across ponds (see Table 4.2) supports the hypothesis that older ponds had more established populations comprised of broad range of ages from 1 to 6-years. Younger ponds (see Table 4.2) appear to be dominated by older fishes, suggesting that older disperse into these novel habitats and require some time to generate a self-sustaining population.

Table 4.7: Life history comparison of *Glossogobius callidus* with other goby species using Von Bertalanffy growth parameters and calculated (phi-prime: Pauly and Munro, 1984).

Species	**Region**		**L_∞ (mm)**	**K (y^{-1})**	**t_0**	**Phi Prime**	**A_{max}**	**A_{mat}**	**Mmax/Amat**	**Mortality rate**	**Main ref**
G. callidus	SA	P	92	0.5	-0.43	0.69	7	2	3.5	0.88	This study
	SA	M	70	1.4	-0.06	0.85	6	2	3	1.06	This study
	SA	F	67	1.8	-0.05	3.9	5	2	2.5	1.38	This study
N. melanostomus	USA	M	215	0.38	-0.83	0.91	1	2	0.5	1.04	1
	USA	F	237	0.22	-1.59	1.72	4	3	0.75	1.3	2; 3
G. niger	Turkey	F	148	0.3	-1.46	1.85	9	2	4.5	0.69	4
		M	167	0.3	-2.21	1.92	9	2	4.5	0.69	4
A. minuta	Italy	M	740	1.41	-0.18	1.89	5	4	1.25	2.46	5
	Italy	F	660	1.61	-0.15	1.85	5	4	1.25	3.01	5
N. fluviatilis	Ukraine		138	0.69	-0.29	2.12	4	1	4	1.31	6
P. lozanoi	Europe		860	0.89	-0.20	1.82	3	1	3	1.46	7
G. paganellus	Isle of Man		116	0.41	-0.2	1.74	3	1	3	1.55	8

1 = MacInnis and Corkum (2000a); 2 = Apanasenko (1973); 3 = Skora *et al*., (1999); 4 = Filiz and Toğulga (2009) ; 5 = La Mesa (1999); 6 = Bilko (1971); 7 = Froese and Binohlan (2003); 8 = Pauly (1978)

The life-history characteristics of *G. callidus* in comparison with data from the literature are summarised in Table 4.7. In comparison to other species for which data were available, *G. callidus* had relatively moderate growth performance, was relatively long lived (7-years vs. maximum of 9-years) and attained maturity at ages comparable to most species. In terms of longevity, data on other gobies suggest that most are sort lived species seldom attaining ages > 4 years. By comparison, *G. callidus* was relatively long-lived (7-years) which was similar to that reported for *G. vittatus* where males attained ages of 7-years (Kovačić, 2006) but shorter lived than *G. niger* from Turkey (to 9-years) (Filiz and Toğulga, 2009).

The growth performance of *G. callidus* was lower compared to *N. melanostomus*, *G. niger*, *N. fluviatilis* and *P. lozanoi* (Apanasenk, 2003; Bilko, 1971; Froese and Binohlan, 2003; Filiz and Toğulga, 2009; and MacInnis and Corkum, 2000a). Populations of *G. callidus* had higher mortality rates compared with the *G. niger* from Turkey (Filiz and Toğulga, 2009). There was no major difference between *G. callidus* mortality rates compared to *N. melanostomus* (MacInnis and Corkum, 2000a) and *N. niger* (Filiz and Toğulga, 2009), which could be another factor contributing to the success of these species, as compared to the high mortalities of *A. minuta* which is supported by the low maximum age of 5-years (Table 4.7), compared to 9-years of maximum age recorded for *N. niger* (Filiz and Toğulga, 2009).

In summary, *G. callidus* is a relatively small sized fish (maximum length of 144.4 mm TL) with intermediate longevity (7-years) and matured at an age that was consistent with maturity of other goby species (within 2-years). This relatively fast growth and early maturity allows juveniles to reach maturity rapidly a trait linked to successful colonisers of novel habitats (Weyl and Hect, 1998). This is typical of other gobies especially the invasive species and this will be further discussed in Chapter 5.

CHAPTER 5

DIET OF *GLOSSOGOBIUS CALLIDUS* (TELEOSTEI: GOBIIDAE) FROM IRRIGATION IMPOUNDMENTS IN THE SUNDAYS RIVER VALLEY OF THE EASTERN CAPE, SOUTH AFRICA

(Based on a manuscript submitted to Journal of Arid Environments)

INTRODUCTION

Understanding the functional role of an organism in its environment is central in ecological investigations (Hughes *et al*., 2009; Jones *et al*., 1994). Resource utilization is important in this regard (Ross, 1986; Sá-Oliveira *et al*., 2014), with food considered one of the most important resources. Information on resource utilization contributes to a greater understanding of interactions among species within an environment and how resources are partitioned and energy is transferred within that ecosystem (Braga *et al*., 2012; Rose, 1986). Feeding studies are, therefore, important for understanding the biology and ecology of a species (Motlagh *et al*., 2012; Verma, 2013). In certain environments, however, basic information on the feeding ecology of species is largely unavailable (Balcombe *et al*., 2005; Kramer *et al*., 2015). The present study investigates the diet of a regionally abundant fish species that has next to no published information on its feeding in freshwater habitats (Wasserman, 2012).

One of the simplest ways to assess food resource acquisition by animals is to do a direct assessment of the stomach contents (Ibrahim *et al*., 2003). This method is particularly prevalent in fish studies as other methods, such as fecal analyses, are not feasible (Hyslop, 1980). Studies of fish diet, feeding ecology and food habits are commonly carried out through dissection and examination of gut contents and the methods of analysis are typically qualitative and quantitative (Cortés, 1997; Hynes, 1950; Hyslop, 1980). While the use of stable isotope and fatty acid analyses are gaining popularity as alternatives to gut content investigations, these methods are more useful for tracking energy flow through ecosystems, typically not providing species-level dietary information (Tierzen *et al*., 1983).

When using quantitative methods, several indices have been used to describe and provide insight into the feeding habits of a predator and the dietary importance of prey taxa. These indices alone can describe the number of individual species found in stomachs (%N), the volumes that each prey item contributes to the overall diet (%V), and how many stomachs a particular prey item was found in (%F).

According to Liao *et al*., (2001), dietary importance based on individual indices can lead to a bias toward a certain prey item. For example, small numerous (%N) and frequently (%F) consumed prey items can be considered to be more important than individuals covering a high volume (%V) and occurring in small numbers. However, prey items occupying more volume (%V) and occurring less frequently among individuals can also be considered more important than small items occurring in high number (%N) (Hyslop, 1980). Therefore, combinations of these indices, such as the index of relative importance (IRI), have proved to be vital as they provide a more balanced view of dietary importance (Cortés, 1998; Liao *et al*., 2001).

The present study assessed the diet of the River Goby, *G. callidus* from irrigation impoundments in the Sundays River Valley. *Glossogobius callidus* are naturally distributed along the eastern seaboard of the South African coastline, between northern Mozambique and the Cape region of South Africa (Wasserman, 2012). In the Eastern Cape region of South Africa, *G. callidus* are particularly abundant in both estuarine and freshwater environments, often dominating fish abundances (Wasserman and Strydom, 2011; Whitfield, 1998).

In the study system, *G. callidus* is native and is currently distributed in the headwaters of all the major tributaries as well as the main river channel and its in-stream impoundments (Weyl et *al*., 2010). In addition, the species has now become established in many off-stream artificial impoundments of the region, through natural colonization, inter-basin transfers or active stocking (Weyl *et al*., 2010; Woodford *et al*., 20013). Despite the abundance of the species, very few studies have assessed the diet of *G. callidus*, with no published information available on the feeding of adult *G. callidus*. The only published information on feeding of the species is limited to research done on early life-history stage individuals from estuarine environments (Wasserman, 2012) and experimental feeding studies (Parkinson and Booth, 2011; Vumazonke *et al*., 2008; Wasserman *et al*., 2014).

The abundance and size of species within an ecosystem can reveal how resources are partitioned and how these resources including space, time, and food influence the structure of fish communities (Corréa *et al*., 2011; Wasserman, 2012; Whitehead *et al*., 2007). The availability and use of resources in an ecosystem by any organism have major influences on the population dynamics of the species (Rose, 1986). Understanding the manner in which large numbers of the same species are distributed in certain environments remains a major challenge towards understanding the ecological mechanism by which these large numbers exist and share resources (Taher, 2010). To understand the dietary specialization of any given group of species, the niche breadth parameter is very important (Sá-Oliveira *et al*., 2014). Dietary specialization of a species is influenced by niche breadth; therefore, niche overlap provides the necessary knowledge to understand the structure of communities (Corréa *et al*., 2011).

Understanding the feeding patterns of species requires knowledge of the prey items that would make-up the diet of the predator (Wetherbee *et al*., 2004). These are mostly mediated by the type and intensity of the interactions between predators and their prey (Savino and Stein, 1989). This study investigates the interaction between terrestrial and aquatic life-history stages that will form part of a larger understanding of the aquatic food-webs. The dynamics of the feeding process must be accounted for and thus to understand the ecological interactions between predator and prey, we must have knowledge of the amount of food ingested and the feeding frequency of the predator. The foraging success of any species is affected by both prey and predator size, therefore understanding the relationship between these remains of paramount importance (Scharf *et al*., 2000). There is no information on *G. callidus* trophic dynamics in these environments and as such, the diet of *G. callidus* was assessed for a range of size classes across seasons.

MATERIALS AND METHODS

Glossogobius callidus was sampled monthly from August 2013 to March 2015, in the Sundays River Valley Irrigation ponds in the Eastern Cape Province of South Africa. (see Chapter 2 for details).

To assess seasonal aspects of dietary intake, representative *G. callidus* samples were collected in all seasons, in the laboratory samples were sorted into five size classes (size class 1 = 20 - 40 mm, 2 = 41 - 60 mm, 3 = 61 - 80 mm, 4 = > 80 - 100 mm Total Length (TL)). Using a dissecting microscope the diet was assessed by removing the entire gut of each examined fish. Each gut was dissected and the contents emptied into a 1 mm deep tray divided into of 1 mm x 1 mm grids. A visual estimate of stomach fullness was made for all *G. callidus* samples and categorised as either empty or contained food items.

Contents were sorted according to the numerical, occurrence, and indirect volumetric method of gut content analysis following recommendation by Hyslop (1980). Gut contents were categorised into their relevant taxonomic classes and identified to the lowest possible taxon using Day *et al*. (2003), de Moor *et al*. (2003a), de Moor *et al*. (2003b), Gerber and Gabriel (2002), and Skelton (2001). For invertebrates, prey counts were based on head counts as their bodies had often been digested. Unidentified organic matter was treated as presence/absence information and was therefore not included in calculations of relative prey abundance as it was not possible to count items individually. An indirect volumetric assessment of the volume of each identified food category was then obtained by flattening food items in the tray under a microscope slide to a depth of 1 mm in thickness (Kramer *et al.,* 2015; Wasserman, 2012). Given that the tray was graduated, the volume of each prey item could be assessed.

Data Analysis

Abundance, frequency, occurrence and percentage volume was calculated for each dietary prey. Sand detritus and stones could not be counted; therefore, they were only recorded as either present or absent. Prey abundance (%N) was expressed as the number of individuals as the percentage of all prey items. Frequency of occurrence (%F) was expressed as the number of stomachs containing a given taxon as a proportion of all sampled stomachs. The percentage volume (%V) of each prey item consumed was expressed as the percentage of the total volume of dietary contents. In addition, the relative importance of each invertebrate taxon was assessed using a modification of the Pinkas *et al*. (1971) index of relative importance (IRI).

$$IRI = (\%N + \%V) * \%\, F$$

For comparative purposes, IRI value for each group was expressed as a proportion of the sum of IRI value calculated for all prey items (%IRI). The (%) of IRI values for each prey item were used for all comparative analyses including the determination of niche breadth. To do this the dietary diversity (Levins niche breadth) equation was used:

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

where Pi is the relative frequency of prey item i, in the diet of the predator P (Levins, 1968). As Pi is the denominator, larger B values represent greater dietary diversity. The modification to calculate standardize niche breadth (scale from 0 – 1) was estimated to Krebs (1989) as follows:

$$Ba = \frac{(B-1)}{(n-1)}$$

where, B_A is Levins standardized niche breadth and n is the number of food items.

As food group compositions in the alimentary canal were determined by size class,ontogenetic dietary shifts could be assessed. In addition to comparisons of %IRI and Levins niche breadth, a community analysis approach was employed whereby the dietary community was contrasted across size classes. %IRI values for each prey item was used for all comparative analyses, used to construct a simple classification tress (Euclidean distance, single link) in Primer v6 software package (Clarke and Warwick, 1994) to examine the similarities between size classes of *G. callidus.* Clusters in the dendogram were assessed using group average hierarchical sorting (Clarke, 1993). The contributions of key taxa towards the differences between these groups were then identified using the SIMPER test (Clarke and Warwick, 1994).

RESULTS

Of all 571 *G. callidus* stomachs, examined 97% contained food items and 3% were empty. Empty stomachs were sampled across all seasons. Analysis of the stomach contents revealed a total of 20 prey items belonging to 10 taxonomic groups, with most identified to a broad taxonomic level (Table 5.1). Among the taxonomic groups, aquatic invertebrates were the most diverse group but while relative contribution of the dietary components varied across all size classes and seasons, key prey items were consistently encountered in all size classes: Diptera, Hemiptera, Trichoptera, Odonata, Cladocera, Copepoda, Hydracarina, Amphipoda, and Mollusca (Table 5.1).

Glossogobius callidus belonging to size class 1 were only encounter in the autumn samples, with size-classes 2, 3, and 4 sampled in all four seasons. In winter, Orthocladiinae were the most important prey items followed by Tanypodinae for size-classes 2 (71% and 27.7%), respectively 3 (54.5% and 37.9%) and 4 (51.9% and 42%) (Figure 5.1a). In spring, however, Chironominae increased in relative contribution, dominating the diets of sampled size classes, and particularly dominant in size class 2 (58.7%). Tanypodids still contributed considerably to all size classes in spring as did the Orthoclanids but, with the exception of size-class 3 where no Orthoclanids were encountered (Figure 5.1b). Chironominae were the most important prey item in the summer season in all of the size classes followed by Orthocladiinae (Figure 5.1c).

Chironominae dominance was more pronounced in summer than in spring for size classes 2 (88.5%), 3 (90.8%) and 4 (39.4%) and there was a general increase in the importance of Orthocladiinae with fish size during this season (Figure 5.1c). Diets of size class 1 and 2 fish sampled from autumn were also respectively dominated by Chironominae (78.1% and 88.7%), although corixidae (14.1%) and Ostracoda (3.3%) featured in size class 1, while Ostracod sp. 1 (7.1%) and Zygoptera (2.2%) featured in size class 2 Autumn fish (Figure 5.1d). Chironominae contributed less to the diets of *G. callidus* size classes 3 (47.7%) and 4 (6.7%) in autumn, with increasing contributions of Tanyponids (14.2% and 8.2%) and Orthoclanids (37.2% and 83.9%) respectively observed for these fish. The only identified fish species that were encountered in the guts were the native conspecific *G. callidus* and the invasive mosquito fish *G. affinis*.

Cluster analysis revealed the presence of three distinct groups at the 40% aggregation level (Figure 5.3). Group 1 consisted of size-classes 1, 2, and 3 from spring, summer and autumn (Table 5.3). Group 2 was, comprised of the larger size-classes 3 and 4 from spring, summer and autumn. Group 3 was comprised of size-classes 2, 3, and 4 from winter and autumn.

SIMPER results revealed that two species independently contributed more than 40% to the dissimilarity between Groups 1 and 3. These included Chironominae (54.12%) and Orthocladiinae (37.96%), which cumulatively contributed to 92.08% of the dissimilarity between these groups. Three prey items Chironominae (41.70%), Orthocladiinae (36.06%) and Tanypodinae (16.04%) contributed more than 10% to the dissimilarity between Group 2 and 3 Dissimilarity between groups 1 and group 2 were was related to three prey items, Chironominae (51.76%), Orthocladiinae (35.90%) and Tanypodinae (5.71%) (Table 5.2).

Niche breadth varied with size class and season and generally decreased between winter and autumn for size class 2, while for size class 3, autumn and spring sampled fish had the broadest niches (Figure 5.3). For size class 4 fish, however, niche breadth was greatest in spring, followed closely by winter and summer. While autumn fish produced the lowest niche breadth for size classes 2 and 4, it was the only season in which size class 1 fish were sampled and produced the greatest niche breadth for size class 3 fish.

Table 5.1: Dietary items identified from various size classes of *Glossogobius callidus*, sampled seasonally from the Sundays River Valley irrigation ponds. Values presented as %IRI (Index of relative importance). Size class 1 = 20 - 40 mm; 2 = 41 – 60 mm; 3 = 61 – 80 mm; 4 = >80 mm body length.

		Winter				**Spring**				**Summer**				**Autumn**			
Prey taxa		1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
Diptera	Tanypodinae	-	27.7	37.9	42.0	-	24.8	8.7	25.5	-	0.0	0.8	3.8	2.7	0.4	14.2	8.2
	Orthocladiinae	-	71.0	54.5	51.9	-	0.3	27.2	35.1	-	3.1	4.5	52.3	0.0	0.5	37.4	84.9
	Chironominae	-	0.0	5.5	4.0	-	58.7	35.1	34.4	-	88.5	90.8	39.4	78.1	88.7	47.7	6.7
Hemiptera	Anisops sp.	-	0.0	0.0	0.7	-	0.0	0.5	2.2	-	0.0	0.0	1.6	0.0	0.0	0.0	0.4
	Corixidae	-	0.0	<0.1	<0.1	-	0.4	0.3	0.1	-	2.0	0.2	0.4	14.1	0.6	0.0	0.0
Tricoptera	Philopotamidae	-	0.0	0.0	0.0	-	0.0	0.0	0.0	-	0.1	<0.1	0.0	0.0	0.1	0.0	0.0
Odonata	Zygoptera	-	0.0	0.0	0.4	-	0.0	<0.1	<0.1	-	0.6	0.0	0.1	0.0	2.2	0.4	0.0
Cladocera	Chydoridae	-	0.0	1.0	<0.1	-	2.6	1.9	0.1	-	0.0	0.0	0.0	0.0	0.1	0.0	0.0
Copepoda	Copepoda	-	0.8	0.1	0.0	-	12.4	0.1	<0.1	-	1.4	0.5	<0.1	0.0	0.2	0.0	0.0

Hydracarina	Hydrachnidae	-	0.0	0.0	0.0	-	0.0	<0.1	<0.1	-	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Amphipoda	Amphipoda	-	0.0	0.0	0.0	-	0.0	0.1	0.0	-	3.0	<0.1	0.0	0.0	0.1	0.0	0.0
Crustacea	Ostracoda	-	0.6	1.0	0.8	-	0.7	27.7	2.3	-	0.0	1.9	1.7	5.2	7.1	0.3	0.0
Mollusca	Ancylidae	-	0.0	0.0	0.0	-	0.0	0.0	0.0	-	0.5	0.3	0.0	0.0	0.0	0.0	0.0
	Lymnaeidae	-	0.0	0.0	0.0	-	0.0	0.0	0.0	-	0.0	0.3	0.0	0.0	0.0	0.0	0.0
Teleostei	*G. callidus*	-	0.0	0.0	0.1	-	0.0	0.1	0.2	-	0.7	0.6	0.7	0.0	0.0	0.0	0.0
	G. affinis	-	0.0	0.0	0.0	-	0.0	0.0	<0.1	-	0.0	<0.1	0.0	0.0	0.0	0.0	0.0
	Fish remains	-	0.0	0.0	0.0	-	0.0	0.0	0.0	-	0.0	0.0	<0.1	0.0	0.0	0.0	0.0
	Otoliths	-	0.0	0.0	0.0	-	0.0	0.0	<0.1	-	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Unidentified†	Detritus	-	1	1	1	-	1	1	1	-	1	1	1	1	1	1	1
	Sand	-	1	1	1	-	1	1	1	-	0	1	0	1	1	1	1
	Stone	-	0	1	0	-	1	1	1	-	1	1	1	1	1	1	1

† = presence/absence data whereby 1 = present and 0 = absent

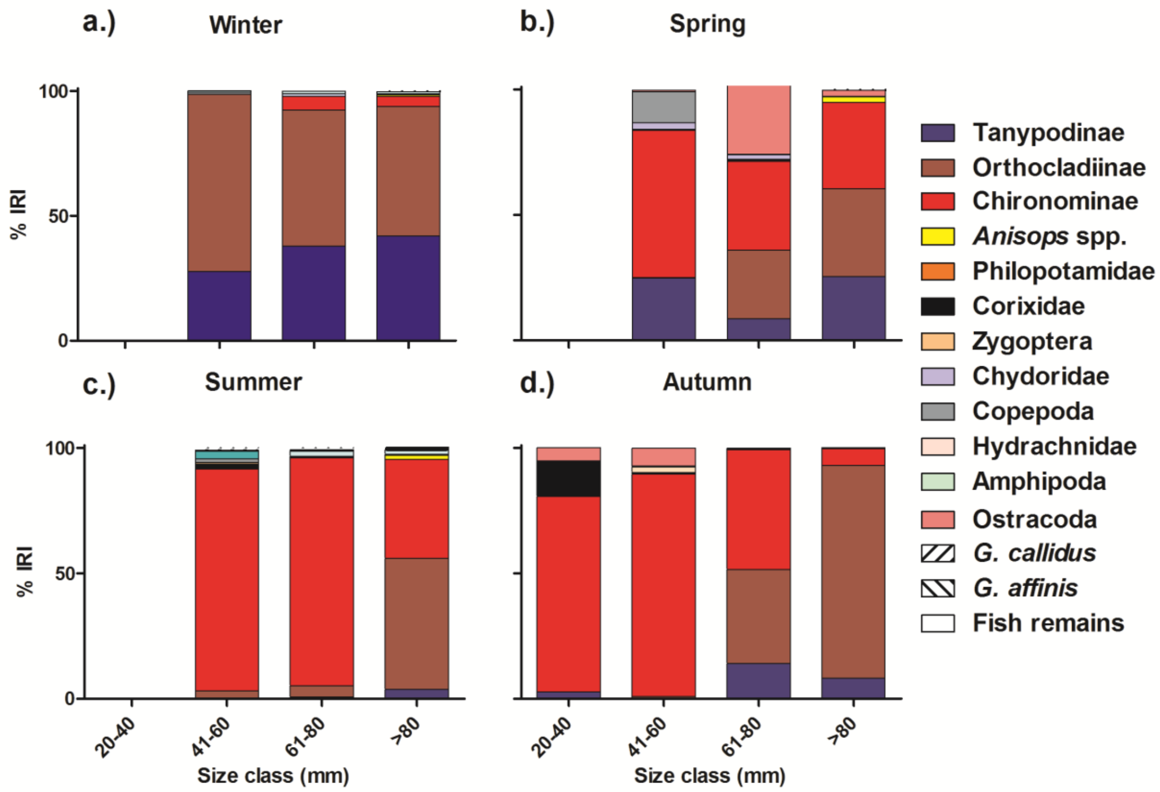

Figure 5.1: Importance rating index (IRI) percentages for each size class of *Glossogobius callidus* sampled from the Sundays River Valley Irrigation ponds during winter, spring, summer, and autumn. n represents the number of prey items in each size class.

Table 5.2: Simper test result for *Glossogobius callidus* groups in the Sundays River Valley irrigation ponds. Group refers to size classes per season as outlined in the similarity dendogram. Group 1 = SP2, AU1, AU2, SU2, SU3; Group 2 = SP3, SU4, SP4, AU3; Group 3 = AU4, WI2, WI3, WI4. Letters refer to season (AU = autumn, SP = spring, SU = summer, WI = winter), numbers refer to size class (1 = 20 – 40 mm, 2 = 41 – 60 mm, 3 = 61 – 80 mm, 4 = >80 mm body length).

Factor	**SIMPER**
	Dominant species (% contribution)
Group 1	Chironominae (47.86%)
	Tanypodinae (30.81%)
	Corixidae (9.56%)
	Copepoda (7.67%)
	Mollusc (2.10%)
Group 2	
	Ostracoda (40.52%)
	Orthocladiinae (28.88%)
	Tanypodinae (20.40%)
	Chironominae (8.53%)
Group 3	
	Orthocladiinae (49.25%)
	Tanypodinae (48.77%)
	Chironominae (1.80%)

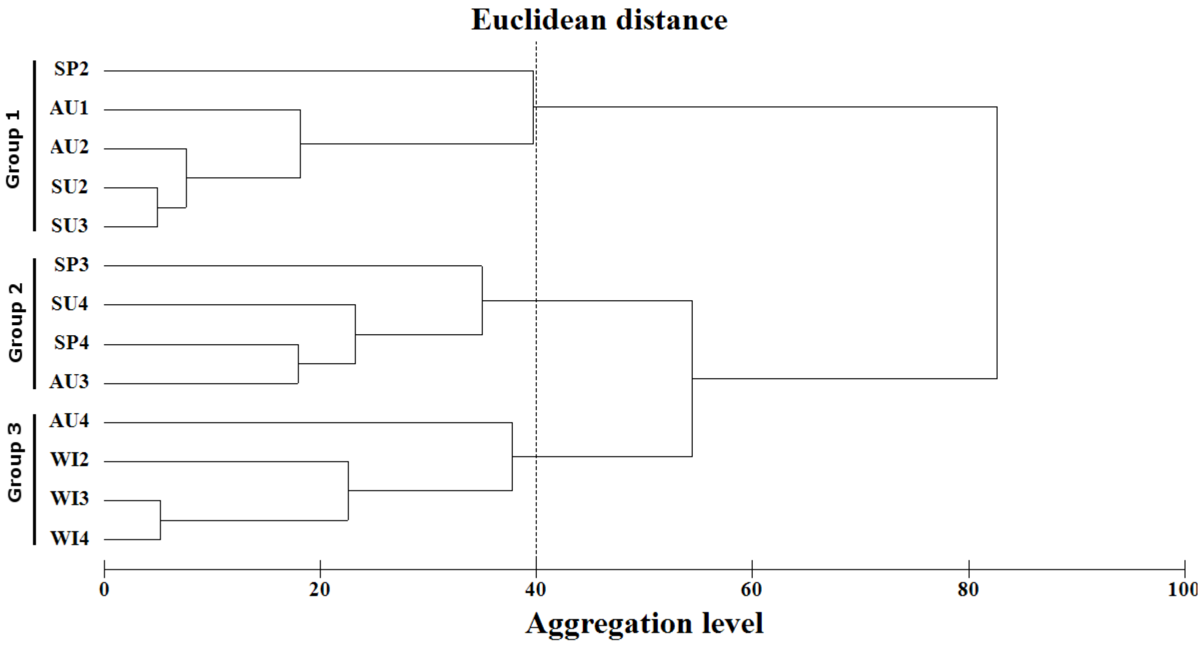

Figure 5.2: Dendogram of dietary similarities (%IRI) of *Glossogobius callidus* size classes per season. Letters refer to season (AU = autumn, SP = spring, SU = summer, WI = winter), numbers refer to size class (1 = 20 – 40 mm, 2 = 41 – 60 mm, 3 = 61 – 80 mm, 4 = >80 mm body length). Cut-off was at the lowest level at which groups are formed using group average.

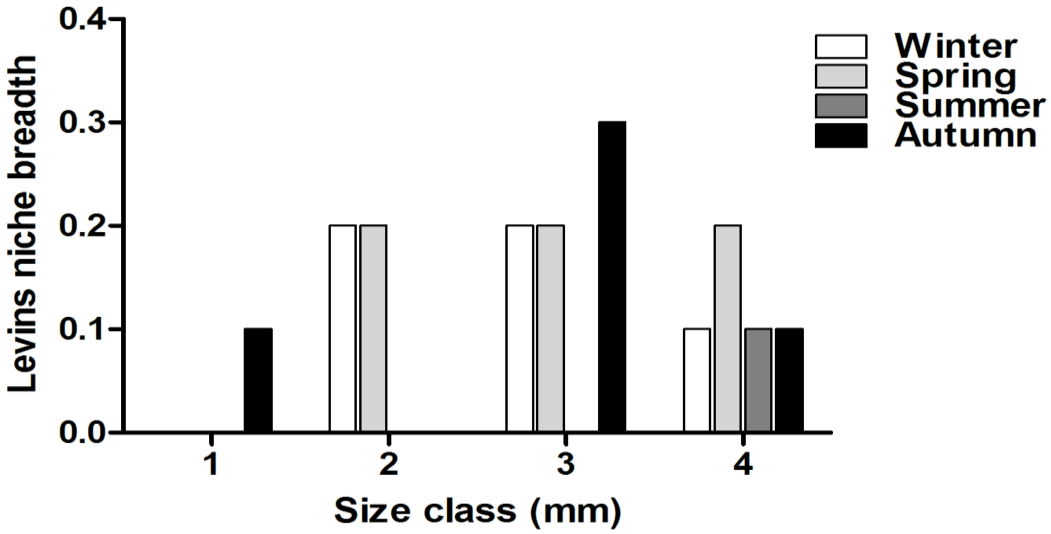

Figure 5.3: Levin’s dietary niche breadth for *Glossogobius callidus* size classes per season size class (1 = 20 – 40 mm, 2 = 41 – 60 mm, 3 = 61 – 80 mm, 4 = >80 mm body length).

DISCUSSION

While there were differences across seasons and size classes, no clear trends emerged, with large degrees of dietary overlap observed across these factors. The one aspect of the study that did stand out was that dipterans dominated the *G. callidus* gut contents irrespective of season and size class in the Sundays River irrigation canals. Given that the larval dipteran stages are epi-benthic and that detritus, stones and sand were also encountered and were likely ingested while foraging, we suggest that the goby is primarily an epi-benthic forager in these habitats even at the early life-history stages. These results are contrary to a previous feeding study on *G. callidus* in a natural environment. Boulle (1990) both found that non epi-benthic and terrestrial invertebrates dominated the diet of *G. callidus* in headwater streams and Wasserman (2012) showed that copepods and amphipods dominated in the lower reaches of rivers.

This highlights the benthic nature of the later life history stages of the species, a trait well observed in the Gobiidae (Corkum *et al*., 2004; Parkinson and Booth, 2011).When considering the findings within the context of larval *G. callidus* diets, that consisted of mostly copepods and amphipods (Wasserman, 2012), it develop likely there is a shift from zooplankton to benthic food sources as fish settle from their planktonic stages.

The diet of *G. callidus* seems to represent that of a generalist feeder with zooplankton contributing significantly to larval diets (Wasserman, 2012) and insects being the primary prey groups of the later life-history stages. Vumazonke *et al*., (2008) found that *G. callidus* mainly consumed gammarid amphipods and harpacticoid copepods through their daily-ratio study, emphasizing the importance of small size fish in the transfer of energy from primary consumers to trophic levels.

There were signs of cannibalism in *G. callidus*, facultative piscivory on the Mosquitofish *G. affinis*. *Glossogobius callidus* and *G. affinis* have been shown to dominate ichthyofaunal communities in the Sundays River irrigation ponds, and while Howell *et al.,* (2013) speculated that *G. callidus* had a negative effect on *G. affinis* through competitive exclusion, the present study suggests that predation may also be contributing to these processes. Elsewhere studies on other gobies, primarily invasive gobies, have showed that these species have a negative effect on native benthic fish communities due to their aggressive behaviour and broad diets (Jude and deBoe, 1996; Kuhns and Berg, 1999). Typical examples include the Round Goby *Neogobius melanostomus* and the Tubenose Goby *Proterorhinus marmoratus,* whose diets commonly include the same taxa as the *G. callidus* observed in the present study, including amphipods, molluscs, and chironomids (Cooper *et al*., 2009; Thomas, 1997).

According to French and Jude (2001), the Round Goby ability to eat a variety of prey items from benthic macro-invertebrates to freshwater fishes has been the major contribution to their devastating impacts on native populations. The manner in which these invasive species have managed to co-exist with other introduced species has been a great phenomenon, French and Jude (2001) pointed out that the Round Goby mainly ontogenetically shifted their diet to mollusc and this further increased with increasing in length.

Wallace and Webster (1996), pointed out the importance of macroinvertebrate in freshwater ecosystems, as they are not only important food source for fishes but also serve as valuable indicators on freshwater ecosystems. What is less clear is the importance of *G. callidus* in structuring invertebrate communities in these irrigation ponds. As ponds regularly receive water from the irrigation canals (Howell *et al*., 2013), there is likely a net import of invertebrates into these systems. Indeed, *G. callidus* population dynamics are considered to be driven by propagules in this way (Woodford *et al*., 2013). However, recent information suggests that there are internal processes likely to be contributing to *G. callidus* population dynamics (Wasserman *et al*., 2015) and, similarly, invertebrate community dynamics are likely to be affected by both internal and external drivers, although this is yet to be assessed.

The Sundays River irrigation canals and ponds form part of larger Inter-Basin Water Transfer scheme (IBWT) making water available for agricultural purposes in the region (Howell *et al*., 2013). Globally, landscape-level alterations such as these are increasing, given the demand for water, particularly in arid regions (Grant *et al*., 2012). Given these changes to the landscape, it is important to understand the ecology of the novel environments as numerous studies found that many terrestrial predators, herbivores, and pollinators have larval aquatic phases (Knight *et al*., 2005) and interactions with freshwater fishes can create trophic cascade that transcend terrestrial and ecosystem boundaries. With the occurrence of fishes also affecting habitat selection, species interactions, and aquatic community structure, and resulting in habitats containing fishes being dominated by small and inactive prey (Johansson and Brodin, 2003, Vonesh *et al*., 2009). Understanding the interactions between aquatic and terrestrial ecosystems can provide crucial information on the effects of anthropogenic environmental changes. Such studies are particularly important in arid environments where novel artificial impoundments are resulting in increased aquatic habitats at the landscape level.

It is, as of yet, unclear how *G. callidus* may structure invertebrate communities in the Sundays River irrigation ponds. As ponds irregularly receive water from the irrigation canals (Howell *et al*., 2013), there is likely a net import of invertebrates into these systems. Indeed, *G. callidus* population dynamics are considered to be driven by propagules in this way (Woodford *et al*., 2013). However, recent information suggests that there are internal processes likely to be contributing to *G. callidus* population dynamics (Wasserman *et al*., 2015) and similarly, invertebrate community dynamics are likely to be affected by both internal and external drivers although this is yet to be assessed.

CHAPTER 6

GENERAL DISCUSSION

Knowledge of the functional traits of fishes (i.e. characteristics of an organism that are linked with its fitness to environmental changes) is the prime goal in fisheries biology to understand how they behave to environmental changes (Mims *et al*., 2010). Fishes occur in different environments, which presents a major ecological challenge to researchers on how they change their life-history traits between a precocial and atricial life styles (Goldstein and Meador, 2004). A number of frameworks have been proposed to characterise species traits (e.g.Balon, 1975, Winemiller and Rose, 1995). These traits offer information with which to interpret the distribution and abundance of species and allow for predictions of how individual species might respond to anthropogenic impacts (Fox *et al*., 2007; Frimpong and Angermeier, 2009; King and McFarlane, 2003). Winemiller *et al*., (2015) considered that adaptation to environmental variation involving aspects such as abiotic and biotic changes, resource availability, population density and challenges to dispersal are merely a suite of inter-correlated functional traits identified from life history traits.

A number of life-history frameworks have been proposed. For example, MacArthur and Wilson (1967) proposed a life-history theory for species with '*r*-selected' and '*K*-selected' traits, their theory predicted that '*r*-selected' trait species would do well in newly established environments because they have traits such as early maturity and high reproductive investment that allow for rapid colonisation. '*K*-selected' trait species would be expected to have lower reproductive investment and greater investment in individual offspring, a strategy that is better for biologically saturated environments where juveniles have to compete for resources with other species. This study provides the life-history traits of *Glossogobius callidus* from irrigation impoundments in the Eastern Cape, South Africa. The knowledge gained from this study provides some understanding of the life-history traits that make this native species a successful coloniser of novel habitats. A summary of the traits assessed for this species in Chapters 3, 4 and 5 is provided, and compared with other species belonging to the family Gobiidae, in Table 6.1.

Table 6.1: Life history comparison of *Glossogobius callidus* and other goby species. (Data from FISHBASE (www.fishbase.org))

G. callidus	**Life-history**	**Other gobies**	**Main ref**
Habitat	Benthic	Typically benthic with preference for rock and sand e.g., *N. melanostomus* and *N. kessleri*.	1; 2; 3; 4
Growth	Fast Max age: 7 Mortality rate: 1.31???	Generally fast Variable age, range 1-9 Variable mortality. Range = xx –yy	Chapter 4
Reproduction	Male parental care Speleophil (Hole nester); Fecundity: 1028 Spawning season: mid-spring & midsummer; Spawning frequency: 3 times; Maturity length: 115 TL; Maturity: 2 yrs	Male parental care Typically guarding clutch tenders (e.g., *N. melanostomus*, *G. niger*, *N. fluviatilis*, *A. Minuta*) and hole nesters (e.g., *N. fluviatilis*, *N. gymnotrachelus*). Fecundity ranges: 774 (*N. fluviatilis*) - 3076 (*G. paganellus*) Spawning season: mid-spring & midsummer; Spawning frequency: 3-4 times; Maturity length: 20.0 TL (*N. melanostomus*); 12.0 TL (*G. niger*); 8.0 TL (*P. lozanoi*); 20.0 TL (*N. fluviatilis*); 6.5 Tl (*A. minuta*); 13.0 TL (*G. paganellus*); Maturity: 3 yrs (*N. melanostomus*); 2 yrs (*G. niger*); 1 yr (*P. Lozanoi*); 1 yr (*N. fluviatilis*); 4 yrs (*A. minuta*); 1 yr (*G. paganellus*)	5; 6; 7
Tropic	Generalist; Macroinvertebrates	Opportunistic generalist: molluscivores (*N. Melanostomus*)	8; 9; 10
Defence & metabolism	More research needed	Hide (*N. Melanostomus*)	8; 11

1 = Adámek *et al.*, (2007); 2 = Jurajda *et al.*, (2005); 3 = Ray and Corkum (2001); 4 = Skelton (2001); 5 = Corkum *et al.*, (2004); 6 = Konečná and Jurajda (2009); 7 = Kovac and Coop (2009); 8 = Borcherding *et al.*, (2013); 9 = Chotkowski and Marsden (1999); 10 = Vumazonke *et al.*, (2008); 11 = Ricciardi and Rasmussen (1998)

In Chapter 3, it was demonstrated that this species had intermediate fecundity with asynchronous oocyte developmentthat according to Wallace and Selman (1981), that is a trait typically found in species that spawn many times during a breeding season (MacInnis and Corkum, 2000b). Elevated GSI revealed an extended spawning season to be over spring and summer month peaking in October and December. Extended breeding season and multiple spawning are traits that that have been attributed for the success invasive goby species globally (Charlebois *et al*., 2001).

The population dynamics of species are reflected on the growth rate, age-at-maturity and mortality (Carlson *et al*., 2006). Using sagittal otoliths for ageing (Chapter 4) the growth of *G. callidus* was determined to be rapid until maturity was attained (at an age of 2 years), similar to goby species in the Northern Hemisphere (MacInnis and Corkum, 2000a) and which has been used to explain the success of invasive gobies in new environments (Sokołowska and Fey, 2011).

Few studies have documented the diet of *G. callidus*. Those that have described the members of the family Gobiidae have described it as opportunistic feeders such as *N. gymnotrachelus* (Kostrzewa and Grabowski, 2003). The results of this study show similar habits to those observed in other dietary studies on the family, as they all have been described in the literature as being benthic and feeding on a wide array of prey items (Skelton, 2001; Thacker, 2003). Using gut content analysis (Chapter 5), *G. callidus* was classified as a generalist feeder, preying on a wide variety of species and targeting those that are most abundant and this shift in the importance of prey items. This was also observed in the diet of *N. melanostomus* in the Northern Hemisphere (Corkum *et al*., 2004). The generalist manner of *G. callidus* allows it to exploit diverse habitats, also making the species robust to changes in food abundance as depletion of one prey species will simply result in *G. callidus* targeting the next abundant and accessible species.

In summary, categorising *G. callidus* under the Winemiller and Rose (1992) life-history framework, this species can be categorised as an equilibrium life-history strategist. A comparison between goby species on the three main axes of the Winemiller and Rose (1992) framework (mortality, maturity and fecundity) is provided in Figure 6.1. From this comparison it is evident that *G. callidus* shares life-history traits with the invasive gobies *N. melanostomus* and *N. fluviatilis* (Figure 6.1), which also mature relatively early, have intermediate fecundity and intermediate mortality rates compared to the other gobies (Figure 6.1).

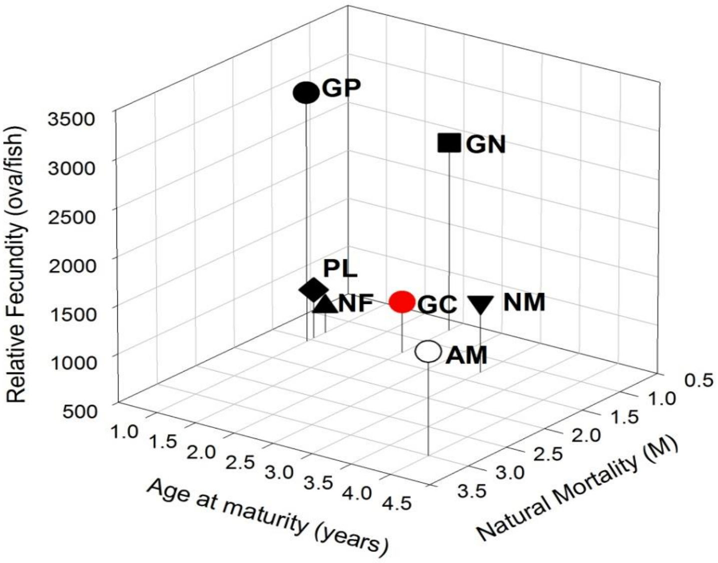

Figure 6.1: Three endpoint life histories for gobies derived from Winemiller and Rose (1992). The endpoint strategies are Relative fecundity, Age at maturity and Natural mortality. GP: *Gobius paganellus*; GN: *Gobius niger*; Pl: *Pomatoschistus lozanoi*; NF: *Neogobius fluviatilis*; GC: *Glossogobius callidus*; Nm: *Neogobius melanostomus*; AM: *Aphia minuta.*

RECOMMENDATIONS FOR FUTURE RESEARCH

Although this thesis was able to contribute to the information on the life-history traits of *G. callidus*, a number of research areas were identified for future research. Among those there is lack of knowledge on the abundance and, seasonal shift in the populations of the prey identified in the stomach contents of *G. callidus*. This makes it difficult to determine the relationship between *G. callidus* and its prey. Future research linking diet with prey abundance could provide insights into prey selection in this species. In this regard, the impacts of *G. callidus* on the insect communities could be studied further to determine how *G. callidus* structures invertebrate communities and to assess for competition between fish species in the ponds. Linking prey intake to reproduction is another interesting field of study as this would help determine how *G. callidus* reproductive behaviour affects food intake, with the aim to compare the diet of males and females during the reproductive period.

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