MOVEMENT PATTERNS OF CAPE STUMPNOSE, *RHABDOSARGUS HOLUBI* (SPARIDAE), IN THE KOWIE ESTUARY, SOUTH AFRICA

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ABSTRACT

Rhabdosargus holubi is an ubiquitous estuary-dependent fishery species endemic to southern Africa. Although aspects of its recruitment dynamics from sea to estuary have received considerable research attention, little is known about its movement behaviour within estuarine systems. Fishery surveys have also revealed that *R. holubi* is a dominant species in catches within several temperate estuaries in South Africa. Therefore, investigating movement behaviour is fundamental to understanding the ecology of this species and providing a means for implementing effective fisheries management strategies.

This study made use of passive acoustic telemetry to quantify the movement patterns of juvenile *R. holubi* in the Kowie Estuary, in the Eastern Cape Province of South Africa. Previous studies have revealed that surgically implanted transmitters may have adverse effects on the physiology and behaviour of tagged fish. Therefore, due to the small size of juvenile *R. holubi*, an *a priori* transmitter-effect experiment was undertaken. This experiment revealed that juvenile *R. holubi* (146-217 mm fork length) were not adversely affected by the surgical implantation of acoustic transmitters (7 mm Ø, 1.6 g out of water) and hence acoustic telemetry was well suited to monitoring the movements of this species.

A 4-month telemetry study evaluated the movement patterns of 21 juveniles (140-190 mm fork length) tagged in three equal batches along the length of the estuary. Specific aspects of their movement included space use patterns, habitat connectivity, and the influences of geophysical cycles and environmental variables.

Tagged fish exhibited high residency, which varied between each release site area. The mean length of estuary used was greater for the upper batch (15.65 ± 6.49 km) than the middle and lower batches (7.36 ± 3.68 and 2.67 ± 2 km, respectively). In terms of habitat connectivity, once the tagged fish left the estuary, they generally did not return. Tagged *R. holubi* spent the majority (83%) of their monitoring periods within the estuarine environment, with the sea (16%) and riverine (1%) environments being utilised to a lesser extent.

Diel and tidal cycles influenced the movement patterns of juvenile *R. holubi*. However, the high levels of residency meant that fine-scale movements were difficult to detect due to the acoustic receiver deployment configuration (i.e. receivers moored one kilometer apart). The influence of environmental variables on the movement of individuals, modelled with general linear mixed models (GLMMs), revealed that the effects of river inflow and water temperature were greatest. Increased river inflow caused individuals to use larger portions of the estuary. Increased water temperature, particularly river temperature, caused individuals to shift their distributions downstream (towards the mouth of the estuary).

This study highlighted the importance of estuarine nursery habitats to *R. holubi*, suggesting that a loss or reduction of healthy estuarine habitats may lead to significant declines in the abundance of this species. Furthermore, extreme resident behaviour suggests that no-take area closures (estuarine protected areas) would be an effective management strategy for this important fishery species.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

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CHAPTER 1

GENERAL INTRODUCTION

Movement is a fundamental characteristic of an animal's life (Nathan *et al.* 2008). Movement behaviour of an animal is dependent on how the animal makes use of available resources, how it is affected by the environment, and how it is affected by inter- and intra-specific interactions (Andersen *et al.* 2008; Pépin *et al.* 2008). Answering questions such as why, how, where and when animals move, and the ecological consequences of this movement are fundamental to understanding animal movement behaviour (Næsje *et al.* 2012).

Understanding the movement patterns and habitat utilisation of a fish species is essential to understanding its ecology, and implementing effective and sustainable management strategies (Meyer *et al.* 2000; Semmens *et al.* 2007; Abecasis *et al.* 2009). However, fish movement behaviour, in particular, is difficult to quantify, due to the challenges associated with underwater observations (Beck *et al.* 2001). Fish movement behaviour is also highly variable with marked differences in the magnitude and speed of movement among and within species (Kerwath 2005). Numerous methods have been used in an attempt to observe, quantify and explain fish movement behaviour. Each provides various positive and negative attributes, therefore choosing a method should be based on the specific objectives of the study (Gillanders *et al.* 2003).

1.1 Methods for assessing fish movement

Conventional tagging, using dart tags, is one of the most widely used methods to study the movements of fish, mainly due to its simple and cost effective nature. Dart tags are small, economical and easy to apply (Zeller and Russ 2000), thus allowing for high numbers of individual fish to be tagged across a wide range of species. Information gathered using this conventional method can be used to study stock status, residency, dispersal rates and migrations of fish (Kohler and Turner 2001; Cartamil *et al.* 2003; Kerwath *et al.* 2005). However, this technique does not allow for the assessment of fine-scale movements, habitat utilisation and space use patterns between the capture and recapture site (Cartamil *et al.* 2003).

Telemetry, on the other hand, provides the opportunity to track and monitor real-time movements of individual fish, providing a more complete picture of its space use and habitat utilisation as opposed to just the capture and recapture positions obtained through conventional dart tagging.

Telemetry can be categorised into three forms: satellite, radio and acoustic. Satellite telemetry data is recovered via orbiting satellites by land-based receivers, and can provide long-term data over large spatial scales (Hussey *et al.* 2015). This method is particularly suited to air-breathing animals (e.g. terrestrial and large marine vertebrates) as the transmitter requires an uninterrupted path to the orbiting satellites (Hazen *et al.* 2012). Satellite transmitters are also relatively large which precludes the use of this technique in smaller animals (Hussey *et al.* 2015). Satellite telemetry is also relatively expensive which may have implications for the sample size of a study.

Radio telemetry relies on the transmission of radio waves from a transmitter to a receiver and is particularly suited to terrestrial and freshwater aquatic animals; it is not well suited to the marine environment as the conductivity of the saline water tends to hinder the transmission of the radio wave (Pincock and Voegeli 2002).

Acoustic telemetry makes use of sound (acoustic) waves to convey data from a transmitter (attached or implanted on/within an animal) to a receiver that logs the presence of the transmitter (Bridger and Booth 2003). This method can provide long-term, high resolution temporal and spatial data that can be collected and correlated with ambient conditions (Hartill *et al.* 2003; Kerwath *et al.* 2005; Childs 2013). Acoustic telemetry overcomes the limitations of satellite and radio telemetry in several ways. Firstly, the ability to continuously track and monitor individual movements (Zeller and Russ 1998). Secondly, it is more cost effective than other techniques (e.g. satellite telemetry). Finally, unlike radio waves used in radio telemetry, the conductivity of saline water does not hinder the transmission of acoustic waves, therefore acoustic telemetry is suitable for the marine environment (Pincock and Voegeli 2002). Acoustic telemetry is thus probably the most effective method to study fish movement in a marine environment.

1.2 Cape stumpnose (*Rhabdosargus holubi*)

1.2.1 Ecology and biology

The Cape stumpnose, *Rhabdosargus holubi*, is an ubiquitous estuary-dependent fishery species endemic to southern Africa. It is one of 41 species within the family Sparidae (50% of which are endemic to southern Africa) that occur along the southern African coastline (Heemstra and Heemstra 2004). It is known to occur from Cape Point to southern Mozambique (van der Elst 1993; Whitfield 1998) (Figure 1.1), with its distribution mainly being influenced by temperature tolerances at the southern and northern extremes (Blaber 1973). The maximum recorded length of *R. holubi* is 45 cm total length (TL) (Smith and Heemstra 1991; van der Elst 1993; Heemstra and Heemstra 2004) and the South African shore angling record is 2.4 kg (SASAA 2012).

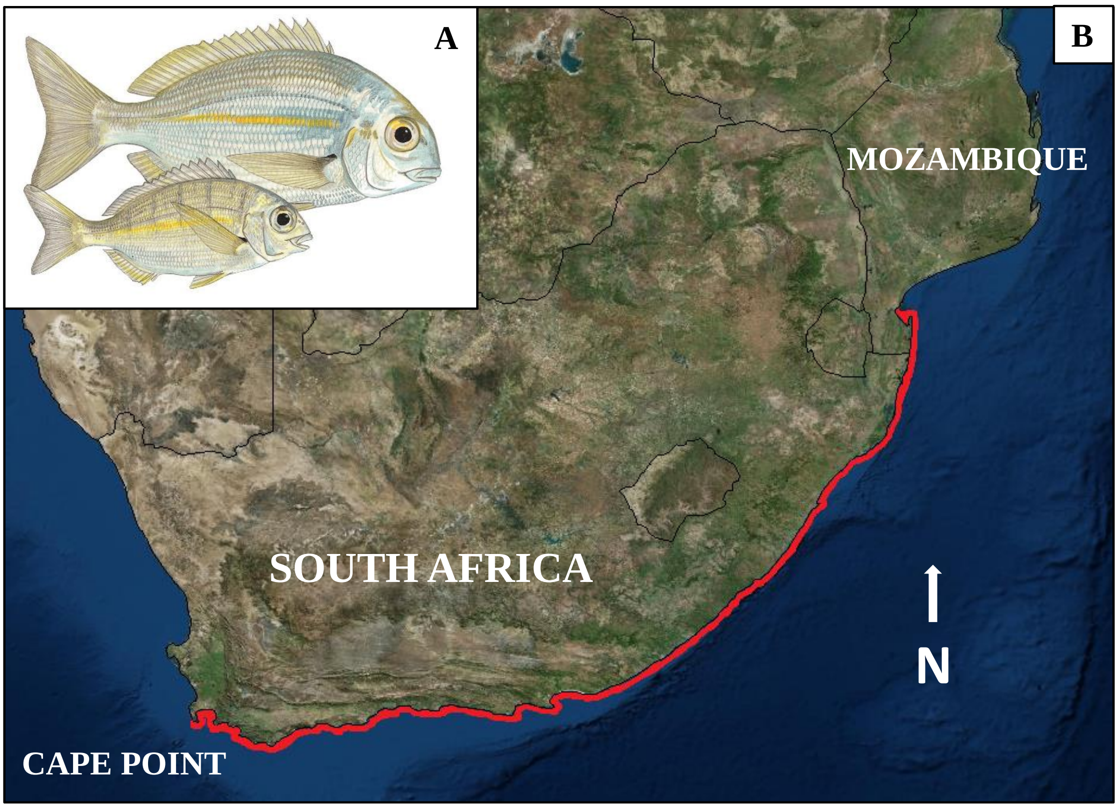

Figure 1.1: A) Cape stumpnose *Rhabdosargus holubi* (Teleostei: Sparidae) as illustrated in Heemstra and Heemstra (2004). B) Distributional range (red zone) of *R. holubi* along the southern African coastline (van der Elst 1993; Whitfield 1998)

Rhabdosargus holubi is one of the most abundant estuary-dependent fish species within the Eastern Cape Province (Vorwerk *et al.* 2001; Harrison 2005) and provides a good example of a marine fish that is highly dependent on estuaries during its life cycle. Apart from a few freshwater fishes, estuary-associated species can be divided into two main categories, based on their ability to breed within the estuarine environment (Whitfield 1990). These include (1) euryhaline marine species that spawn exclusively within the coastal zone and (2) resident estuarine species which spawn within the estuarine environment (Whitfield 1998). *Rhabdosargus holubi* forms part of the first category.

Adult *R. holubi* spawn in the nearshore environment (Wallace 1975a). It is a serial spawner and has an extended breeding season, although different spawning peaks have been observed across its distributional range corresponding to changing temperature and rainfall patterns along the coastline (James *et al.* 2007a). In KwaZulu-Natal and the northern Eastern Cape, peaks in spawning occur during late winter and spring (Wallace 1975b). In the southern part of the Eastern Cape and Western Cape, spawning peaks occur from spring to late summer (Blaber 1973; Wallace 1975b; Beckley 1983). In Eastern Cape and Western Cape surf zone waters, the early postlarvae of *R. holubi* is one of the most abundant species in terms of the ichthyoplankton community, with peaks in abundance occurring during winter (Cowley *et al.* 2001) and spring/summer (Whitfield 1989a) in the respective surf zones.

At the postlarval developmental stage, *R. holubi* uses olfactory cues to locate and recruit into estuaries at a standard length <15 mm (Blaber 1973; Beckley 1985a; James *et al.* 2008b). Within permanently open estuaries (POEs) the recruitment of postlarvae occurs mainly during the flood tide (Beckley 1985a). Within temporarily open/closed estuaries (TOCEs), recruitment occurs mainly following mouth opening events, as well as during overwash events under closed mouth phase conditions (Whitfield 1989b; Cowley and Whitfield 2001a; Cowley *et al.* 2001). The combination of an extended breeding season, serial spawning, and the ability to make use of overwash and mouth opening events have been proposed to contribute to a life history strategy that prevents recruitment failure and accounts for the high abundance of *R. holubi* in most TOCEs (Cowley 1998).

Juvenile *R. holubi* are entirely dependent on the estuarine environment for at least the first year of their lives and remain in these systems until just prior to the onset of sexual maturity (Blaber 1973). The estuarine environment can be subject to extreme changes in environmental conditions over short periods of time; therefore it is important for an estuary-dependent species to be able to tolerate a wide range of environmental conditions, particularly salinity and temperature. Juvenile *R. holubi* are extremely tolerant of salinity fluctuations and are able to survive and maintain their osmotic balance in salinities ranging from 1 to 65 (Blaber 1973). They are also able to tolerate a relatively wide temperature range, from 10 ˚C to 30 ˚C (Blaber 1973).

During the period within the estuarine environment, juvenile *R. holubi* usually form a close association with submerged macrophyte beds which they use as a source of food and a refuge against predation (Beckley 1983; Heemstra and Heemstra 2004). In estuarine areas where macrophyte beds (particularly *Ruppia spiralis, Potamogeton pectinatus* and *Zostera capensis*) are extensive, juveniles can be found in shoals of up to 400 individuals (Blaber 1973). In the Swartkops Estuary (Eastern Cape), Beckley (1983) found that *Z. capensis* beds were of particular importance to juvenile fish species, with *R. holubi* being the second most abundant. In the Kromme Estuary (Eastern Cape), Hanekom and Baird (1984) found that there were significantly more juvenile *R. holubi* in areas with *Zostera* beds than areas without. In the Swartvlei and East Kleinemond estuaries (Eastern Cape), *R. holubi* abundance declined after mass losses of submerged aquatic macrophytes (Whitfield 1984; Sheppard *et al.* 2011), further illustrating their importance.

Rhabdosargus holubi is a dominant marine species in many of the Eastern and Western Cape estuaries and is able to reach relatively large population sizes. Netting data resulted in population estimates of 13 653 to 110 485 individuals in the relatively small East Kleinemonde Estuary (TOCE) in 1994 and 1995, respectively (Cowley and Whitfield 2001b), contributing approximately 80% to the total nettable population of marine fishes in the estuary. Similarly, the *R. holubi* population in the West Kleinemonde Estuary (TOCE) during 1971 and 1972 was estimated at 13 000 to 55 000 individuals. Several studies have shown that POEs generally have a lower abundance of juvenile *R. holubi* when compared to TOCEs, probably due to the relative scarcity of submerged aquatic macrophyte beds in most POEs (Hanekom and Baird 1984; Whitfield 1989b; Vorwerk *et al.* 2003). Similarly, Whitfield *et al.* (1994) attributed the higher densities of juvenile *R. holubi* in the permanently open Kowie Estuary versus the permanently open Great Fish Estuary to the extensive coverage of *Zostera* beds in the former estuary and their absence in the latter.

Juvenile *R. holubi* feed mainly during daylight hours, but do exhibit considerable flexibility in terms of their feeding patterns (Blaber 1973; De Wet and Marais 1990). When available, macrophytes form the bulk of their diet but are usually not assimilated and pass through the gut unaltered (Blaber 1973). Epiphytes and organisms attached to the macrophytes are digested and generally constitute their main food source (Blaber 1973). However, under certain conditions submerged macrophytes are assimilated. Carassou *et al.* (2016) showed that in freshwater environments, when preferred food sources were scarce, macrophytes contributed more than 60% of their diets. Amphipods, isopods, bivalves, gastropods and polychaetes are also important dietary items (Blaber 1973). In the East Kleinemonde Estuary, Sheppard *et al.* (2012) by means of stable isotope analyses using $\delta^{13}C$ and $\delta^{15}N$ signatures showed that epiphytic algae were a significantly more important source of nutrition to *R. holubi* than the dominant submerged macrophytes *R. cirrhosa* and *P. pectinatus*.

During periods of relatively low macrophyte abundance, dietary shifts can occur. In the West Kleinemonde Estuary, Blaber (1973) showed that invertebrates dominated the diet of *R. holubi* during low abundance periods of *R. spiralis* (i.e. in 1971), while this particular macrophyte dominated in the diet during high algal abundance periods (i.e. in 1972). Although juveniles are omnivorous with a marked preference for plant material, adults tend to be more predatory (Buxton and Kok 1983). This ontogenetic shift in diet is associated with differences in teeth morphology between juvenile and adult *R. holubi*. Adult molariform teeth are more robust and better suited to crushing shelled invertebrates, whereas the sharp tricuspid juvenile teeth are better suited to cropping plant material (Blaber 1973).

Studies have shown that the annual increase in length of individuals ranges from 60 to 100 mm (Blaber 1973; Wallace and van der Elst 1975), with sexual maturity attained at 190 to 200 mm (TL) (Heemstra and Heemstra 2004). Before the onset of sexual maturity, large juvenile *R. holubi* migrate back to sea and generally do not return to the estuary (Blaber 1973; Beckley 1983; Whitfield 1998). Adults almost exclusively inhabit the coastal marine environment in water <50 m depth and are often associated with rocky reefs.

1.2.2 Movement behaviour

The movement behaviour of *R. holubi* along the southern African coastline is poorly understood, emphasised by the low recapture rate recorded for *R. holubi* in the

Oceanographic Research Institute (ORI) national tagging programme (Gotz and Cowley 2013). The intent of this program (commenced in 1984) was to enlist active angler support in the tagging of specified species around the entire southern African coastline with the aim of providing information on the migration routes, growth rates, stock identity and population dynamics of important line-fish and elasmobranch species (Dunlop and Mann 2014). Since 1984, only 8 of 1 087 tagged and released individuals have been recaptured (Dunlop and Mann 2014). The longest distance between the capture and recapture sites of an individual is 56 km (Dunlop and Mann 2014). The low recapture rate for *R. holubi* suggests that conventional external fish tags are not an effective method for monitoring adult movement patterns of this species.

1.2.3 Rhabdosargus holubi fishery

Factors threatening fish in estuarine environments vary and change from one area to another. However, consumptive exploitation has been identified as one of the most important threats to South African estuary-associated fishes (Whitfield and Cowley 2010). Furthermore, the banning of off-road vehicles from South African beaches (December 2001) has led to an increase in fishing effort in estuaries which has led to further pressure being exerted on estuary-associated species (Potts *et al.* 2005).

Although *R. holubi* is of little commercial value due to its small size, a number of fisheries resource surveys conducted in South African estuaries have shown it to be important in recreational and subsistence catches. Catch data recorded by fisheries law enforcement personnel from January 1996 to April 1997 revealed that *R. holubi* dominated catches in the Bushmans (45%), Swartkops (36%), Kowie (33%) and Kromme (29%) estuaries in the Eastern Cape (Pradervand and Baird 2002). Furthermore, fishery surveys showed that *R. holubi* is a dominant component of the catches in the Sundays (30%, Cowley *et al.* 2013), Knysna (61%, Napier *et al.* 2010) and Kowie (62%, Cowley *et al.* 2004) estuaries.

These studies highlight that *R. holubi* is a dominant catch species in estuarine fisheries within the Eastern and Western Cape. This dominance in recent years has been attributed to the decrease in abundance of larger estuary-associated species whose stocks have been depleted or have collapsed (Pradervand and Baird 2002). Conservation management should be aimed at the most vulnerable life history stage of a fish species (Wallace and van der Elst 1975). Therefore, due to its significant estuarine dependence, conservation measures for *R. holubi* should be aimed at the juvenile stage.

1.3 Aims and objectives

Although considerable research attention has been placed on aspects of the recruitment dynamics of *R. holubi* from sea to estuary (Beckley 1985a; Cowley *et al.* 2001; James *et al.* 2007c; Reddy *et al.* 2011) and their subsequent association with submerged macrophyte beds (Blaber 1973; Beckley 1983; Sheppard *et al.* 2011, 2012), little is known about the movement patterns of the juveniles within estuarine systems. Therefore, the overall aim of this study was to gain a better understanding of the spatial ecology of juvenile *R. holubi* within the Kowie Estuary, South Africa. Making use of acoustic telemetry, the movement patterns of juvenile *R. holubi* were monitored to determine the degree of residency within the estuary and/or connectivity between the estuary, river and marine environment, as well as to understand the abiotic factors influencing their movement patterns within the estuarine environment.

While acoustic telemetry is an effective tool to study the movement patterns of fishes, the interpretation of the results relies on the assumption that tagged fish are not affected by the method of capture, surgical implantation and subsequent presence of the transmitter (Jepsen *et al.* 2002; Bridger and Booth 2003). Previous studies have shown that the transmitter presence may negatively affect the behaviour and physiology of a fish (Marty and Summerfelt 1986; Greenstreet and Morgan 1989). Therefore, prior to the telemetry study, a transmitter-effect experiment was necessary to test the effects of surgical implantation of acoustic transmitters on juvenile *R. holubi*.

The specific objectives of this study were to:

1) Determine the effects of the surgical procedure, implantation, and subsequent presence of the transmitters on juvenile *R. holubi*.
2) Quantify the space use and habitat connectivity of juvenile *R. holubi* within the Kowie Estuary.
3) Identify the geophysical cycles and environmental variables that influence the movement patterns of juvenile *R. holubi* within the Kowie Estuary

CHAPTER 2

GENERAL METHODS AND MATERIALS

2.1 Acoustic telemetry

Acoustic telemetry is rapidly becoming the most popular technique to study the movement behaviour of fishes, in particular aspects of fish ecology including migrations, habitat utilisation, activity patterns, home range use and the homing ability of fishes (Hart and Summerfelt 1975; Stasko and Pincock 1977; Giacalone *et al.* 2005; Hussey *et al.* 2015). The ability to continuously monitor fish in their natural environment using this method allows for new insights into the abiotic and biotic factors which influence fish movement behaviour (Childs *et al.* 2008a; Hindell *et al.* 2008). Furthermore, information from telemetry studies has been used to understand the effects of habitat degradation, habitat loss and environmental change on species, as well as to make estuarine and marine management decisions, and effectively design protected areas (Garshelis 2000; Spedicato *et al.* 2005; Taylor *et al.* 2006).

Over the past decade the number of aquatic acoustic telemetry studies have increased six-fold, spanning all continents (primarily in developed countries) (Hussey *et al.* 2015). Almost 500 teleost and over 100 elasmobranch species have been tracked, with the majority of research focusing on estuarine, coastal and freshwater systems (Hussey *et al.* 2015). Within southern Africa, acoustic telemetry has been used to study the movement patterns of several fish species, including dusky kob *Argyrosomus japonicus* (Cowley *et al.* 2008), spotted grunter *Pomadasys commersonnii* (Næsje *et al.* 2007), white steenbras *Lithognathus lithognathus* (Bennett *et al.* 2015), shad/elf *Pomatomus saltatrix* (Hedger *et al.* 2010), white stumpnose *Rhabdosargus globiceps* (Attwood *et al.* 2007) and leervis/garrick *Lichia amia* (South African Institute for Aquatic Biodiversity (SAIAB) unpubl. data).

Acoustic telemetry, however, does have two main constraints. Firstly, the high cost of the equipment (although not as high as satellite telemetry) used for telemetry studies generally only allows for a small sample size compared with other inexpensive techniques, such as conventional dart tagging (Hartill *et al.* 2003; Kerwath *et al.* 2005; Childs 2013). Zeller and Russ (2000) recommended that due to the high cost of acoustic telemetry a combination of transmitter-equipped fish with a large sample of dart-tagged fish would be best suited to obtain movement data. Secondly, the duration of the study period is restricted by the battery life of the transmitters (i.e. the larger the transmitter the longer the battery life, hence the longer the duration of the study) (Heupel *et al.* 2006). Transmitter size also has implications for the size of fish that can be tagged. Smaller fish require smaller transmitters, which are restricted by shorter battery lives, therefore long-term studies of small-bodied fish as well as movement and migrations throughout fish ontogeny are precluded (Hartill *et al.* 2003).

Acoustic telemetry can be divided into two main approaches for tracking fish movement: passive tracking and manual tracking. Manual acoustic tracking involves the active monitoring of an individual fish from a vessel, using a mobile receiver. The mobile receiver is used to determine the direction in which the signal is transmitted, and the strength of the signal acts as a proxy for distance from the tagged fish. Manual tracking provides accurate, high-resolution positional information as well as fine-scale temporal and spatial movement data, which can be used to estimate home range size, shape and location (Bennett 2012). Manual tracking also allows for the *in situ* recording of environmental parameters (e.g. salinity, temperature, turbidity, and substrate type) in order to determine which environmental factors influence movement behaviour and to identify important habitats (Nathan *et al.* 2008; Abecasis *et al.* 2009). Manual tracking does have several disadvantages. It requires more resources (e.g. a vessel and skilled personnel), is more labour intensive, and long hours of tracking are required to collect a limited amount of data, making long-term studies unfeasible (Hedger *et al.* 2010). Only a single individual can be monitored at a time, and contact with that specific individual must be maintained for an extended period in order to collect movement data (Heupel *et al.* 2006). The tracking vessel is required to remain within close proximity of the subject, which may influence the behaviour of the individual and bias the data collected (Heupel *et al.* 2006). Manual tracking may not be possible when ambient conditions are unsafe for a researcher to be in the field (Heupel *et al.* 2006).

Passive tracking involves the monitoring of fish using an array of stationery automated data-logging receivers which record the date and time of unique signals received from individual transmitters. This method is ideal for studying site fidelity and long-term survivorship, as well as cyclical movement patterns such as diel, tidal and seasonal movements (Heupel *et al.* 2006; Bellquist *et al.* 2008). Within estuarine systems it has been used to determine connectivity between environments (e.g. riverine, estuarine and marine) as well as the proportion of time spent within these different environments (Childs *et al.* 2008a; Hindell *et al.* 2008). Passive tracking requires the initial deployment of the receivers and the subsequent implantation of transmitters into the desired subject. Data collection by the receivers is automated, except for the uploading of the data stored on each receiver. It is less labour intensive, allows multiple individuals to be tracked simultaneously, and its remote nature means that individuals can be monitored without their natural behaviour being affected by observer presence (Winter 1996). Passive tracking also allows for the uninterrupted logging of data, regardless of ambient conditions (Heupel *et al.* 2006). Passive tracking does, however, have a few disadvantages. Passive tracking has an omni-directional detection range, therefore high resolution, positional information on individual fish is not possible using an array of independent receivers (Heupel *et al.* 2006). This method also requires a fish to remain within the detection range of the receiver array in order for data to be collected (Simpfendorfer *et al.* 2002).

Based on the advantages of passive acoustic telemetry this technique was deemed suitable to answer the key research questions and objectives for this study. Therefore, this study made use of passive acoustic telemetry to quantify the movement patterns of juvenile *Rhabdosargus holubi* in the Kowie Estuary, in the Eastern Cape Province of South Africa.

2.2 The Kowie Estuary

The Kowie Estuary is representative of a typical warm-temperate, permanently open estuary, and harbours an abundance of juvenile *R. holubi* (Heinecken and Grindley 1982; Cowley *et al.* 2004). Although the lower reaches are impacted on by anthropogenic activities, the Kowie Estuary is deemed to be in fair condition (Whitfield 2000). Apart from a weir situated approximately 500 meters upstream of the tidal limit (approximately 22km from the mouth), there are no physical structures which would block the movement of fish within the estuary. For these reasons it was chosen as the study site for the telemetry study. This section will describe the physical and biological characteristics of the Kowie Estuary.

2.2.1 Physical characteristics of the Kowie River catchment and Estuary

The Kowie River is approximately 70 km in length and flows into the Indian Ocean at the town of Port Alfred, Eastern Cape (33° 36′ S; 26° 54′ E) (Figure 2.1). It is considered perennial, although the flow can be non-existent for 2 to 3 months during abnormal drought conditions (Whitfield and Wooldridge 1994). The region within which the Kowie River is situated has a temperate climate and its catchment receives a mean annual rainfall of 638 mm (Day 1981).

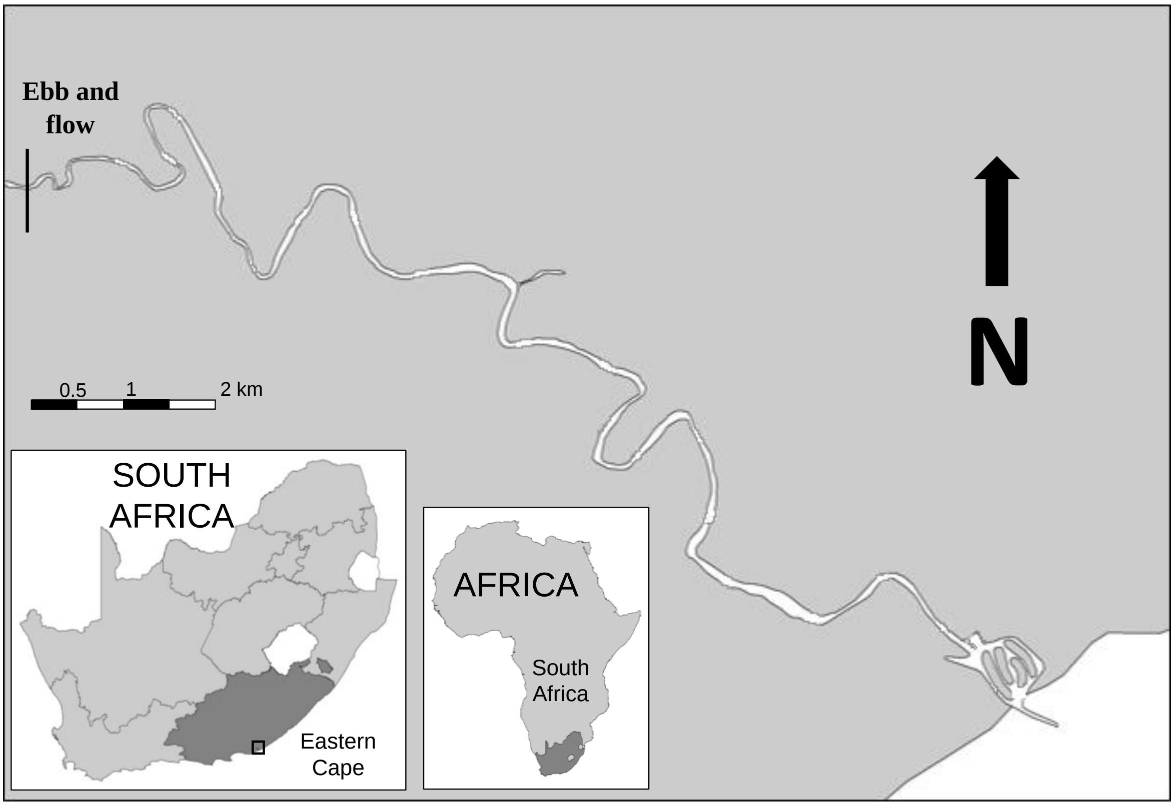

Figure 2.1: Location of the Kowie Estuary in the Eastern Cape, South Africa.

Only 25% of South Africa’s 250 estuaries are permanently open to the sea, of which the Kowie Estuary is one (Whitfield 1998). It is tidal for 21 km with the upper reaches being relatively narrow (50 m) and characterised by steeply sloped banks, which are predominantly vegetated down to the water level. The middle reaches widen to between 100 and 150 m where intertidal mudbanks become more prominent. The lower reaches consist of an artificial channel that is approximately 80 m wide. The actual mouth consists of two breakwaters 75 m apart which extend out through the surf zone (Table 2.1).

Table 2.1: Physical characteristics of the Kowie River catchment and Estuary.

Characteristics (Units)	
Catchment size (km^2)	576-769
Mean annual runoff (m^3)	23×10^6 to 46×10^6
Freshwater inflow (m^{3}s^{-1})	<1
Estuarine length (km)	21
Estuarine width range (m)	50-150
Estuarine surface area (ha)	120
Estuarine depth range (m)	3-10
Tidal range for spring tide (m)	1.7
Tidal flow rate (cm/s)	25

Adapted from Hill (1975), Day (1981) and Heinecken and Grindley (1982), .

Water temperature within the Kowie Estuary varies seasonally. The temperature ranges from 11 to 27 ˚C in the upper reaches and 14 to 22 ˚C in the lower reaches (Day 1981). However, the temperature in the lower reaches may drop below 14 ˚C during marine upwelling events (Bok 1983). Daily fluctuations in water temperature also occur due to the tidal movement of sea water into the estuary. The average salinity is usually just above 30, however this may vary between 40 during drought conditions and 0 during flood conditions (Day 1981). Seasonal fluctuations in salinity have also been shown to occur, ranging from 25 to 35 in summer, 12 to 35 in spring, 17 to 35 in autumn and 21 to 35 in winter (Kruger and Strydom 2010).

The lower reaches of the Kowie Estuary are situated within the town of Port Alfred and are subjected to direct and indirect anthropogenic manipulation (Sale *et al.* 2009; Kruger and Strydom 2010). The main modification to this area was the development of the artificial channel, which first started in 1836 (Turpin 1964), and subsequently a marina and small-craft harbour. This removed large wetland and mudflat areas within the lower reaches (Heinecken and Grindley 1982). The development included the straightening of the main channel, which was diverted to the western side of the original flood plain, and the construction of the east and west breakwaters which extend past the surf zone and give protection to the mouth area (Schumann *et al.* 2001). The last alteration was the extension of the west breakwater by 65 m between 1938 and 1941 (Schumann *et al.* 2001).

The artificial channel is characterised by steep, stone-packed walls sloping into the estuary (Kruger and Strydom 2010). The marina branches off on the east side of the main channel into three smaller channels near the estuary mouth (Kruger and Strydom 2010). Private waterfront homes are a major land use within the marina and along the banks of the main channel of the lower reaches with many houses having jetties and slipways which protrude into the main channel (Sale *et al.* 2009). Upstream from the town the majority of the land adjacent to the estuary is privately owned and used for agriculture particularly the production of beef cattle (Heinecken and Grindley 1982; Cowley and Daniel 2001).

The primary anthropogenic use of the estuary itself is recreational (Heinecken and Grindley 1982). Recreational activities include fishing, sailing, water-skiing, power-boating and rowing (Heinecken and Grindley 1982; Adams 2001). Road access along the course of the estuary is considered good with approximately 25 public access points (Sale *et al.* 2009). Commercial offshore fishing boats make use of the estuary as a harbour and there is also a subsistence fishery which makes use of the estuarine resources (Sale *et al.* 2009).

2.2.2 Environmental parameters during study period

Temperature loggers (Hobo® Water Temp Pro V2), moored alongside three deployed acoustic receivers (Receiver 2, 13 and 22; see Figure 2.7), monitored the water temperature in the lower, middle and upper estuarine regions throughout the telemetry study. Water temperatures during the study ranged from 11.5 to 25.0 °C (mean = 18.5 ± 2.7 °C), 16.5 to 25.7 °C (mean = 21.4 ± 1.8 °C) and 19.0 to 29.7 °C (mean = 24.5 ± 2.0 °C) at Receiver 2, Receiver 13 and Receiver 22, respectively (Figure 2.2).

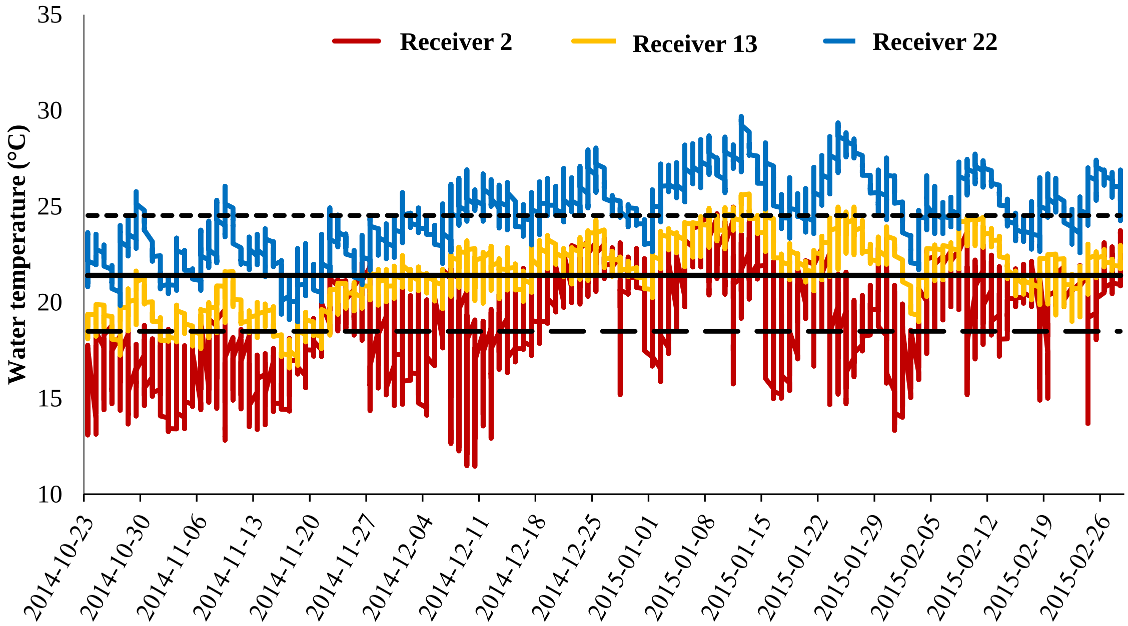

Figure 2.2: Water temperature recorded for the duration of the telemetry experiment (23 October 2014 – 28 February 2015) at three sites: Receiver 2 (lower), Receiver 13 (middle) and Receiver 22 (upper). The black lines illustrate the mean water temperatures for the entire experiment for each region: lower = dashed line; middle = solid line; upper = dotted line.

Environmental data (air temperature, air pressure, and daily rainfall), collected from the Port Alfred airport weather station (-33.5590; 26.8810), were obtained from the South African Weather Service (www.weathersa.co.za). Mean daily atmospheric pressure during the telemetry experiment was 1004.7 (± 4.4) hPa. The minimum and maximum mean daily barometric pressure was 995.3 hPa and 1016.6 hPa (Figure 2.3). Mean daily air temperature during the telemetry experiment was 19.8 (± 2.4) °C. The minimum and maximum mean daily air temperatures were 14.3 °C and 26.7 °C, respectively (Figure 2.3). Total rainfall over the telemetry study was 171.4 mm and at least 0.2 mm of rain fell during 38 out of the 128 days of the experiment (Figure 2.3). The three highest rainfall events recorded on a single day were 23.2 mm (2 February 2015), 22 mm (12 November 2014) and 21.4 mm (24 November 2014). The longest period without rain was 14 days from the 13 December 2014 to 26 December 2014. Mean daily river inflow was 0.42 (± 0.4) $m^3.s^{-1}$, with a range of 0.08 to 2.43 $m^3.s^{-1}$.

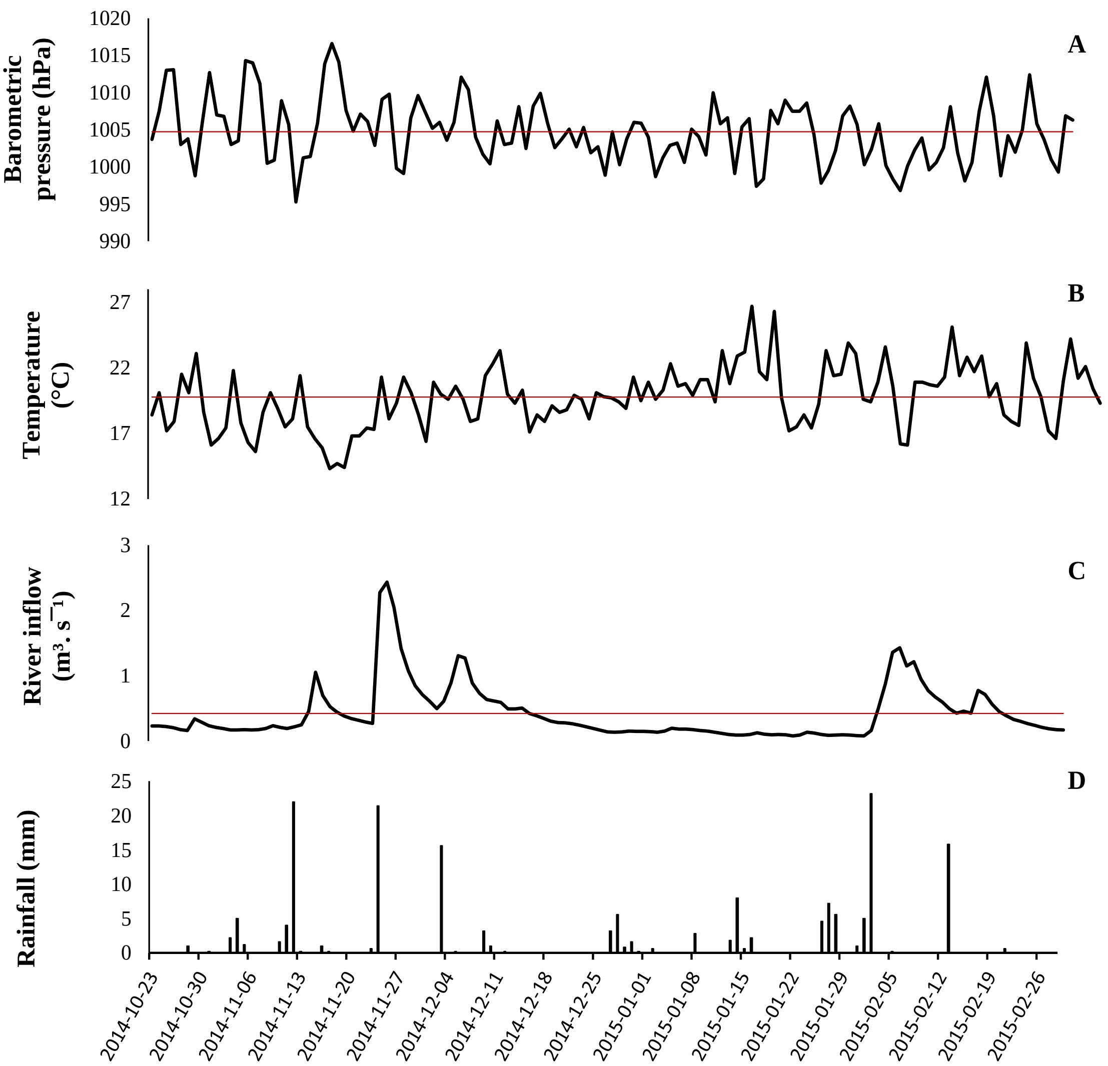

Figure 2.3: Physical parameters recorded for the duration of the telemetry experiment at the Port Alfred airport weather station (-33.5590; 26.8810). A) Mean daily barometric pressure (hPa); B) mean daily temperature (°C); C) mean daily river inflow (m^3. s^{-1}); D) daily rainfall (mm). The red lines indicate the mean of each parameter over the entire experimental period.

2.2.3 Biological characteristics of the Kowie Estuary

Numerous species of phytoplankton, zooplankton, and other flora and fauna have been described and identified within the Kowie Estuary.

Diatoms and dinoflagellates make up the two most common groups of phytoplankton within the Kowie Estuary with Giffen (1970) identifying at least 280 species of diatoms alone. *Ulva capensis*, *Enteromorpha prolifera*, and *Gelidium pristoides* make up the dominant algal species with *Chodria* species, *Centrocerus clavulatum* and *Griffithsia* species less conspicuous (Heinecken and Grindley 1982). A more recent studie observed 89 species of benthic diatoms in the Kowie Estuary with *Entomoneis paludosa*, *Nitzschia reversa*, *N. closterium*, *Pleurosigma elongatum*, *P. salinarum*, *Staurosira elliptica*, *Surirella brebissonii*, and *S. ovalis* being the numerically dominant (Dalu *et al.* 2015). Blue-green algae has also been found in the lower reaches indicating the possibility of high nutrient levels. *Ruppia spiralis* and *R. maritima* are the most dominant aquatic macrophytes and are present in all lagoons and adjacent to the banks. There are patches of *Zostera capensis* but these have been greatly reduced (Day 1981). Inter-tidal macrophytes are represented by *Scirpus maritimus* and tidal salt marshes consisting of *Spartina capensis, Chenolea diffusa, and Sarcocornia perennis* among others.

At least 39 zooplankton species have been identified in the Kowie Estuary of which copepods *Acartia natalensis* and *Pseudodiaptomus hessei* are the most dominant (Heinecken and Grindley 1982). Day (1981) reported a paucity in benthic invertebrate fauna apart from a few dominant species. In particular burrowing mud prawn *Upogebia africana* is extremely abundant in the middle reaches with densities of up to 600 individuals per m^2 recorded. Mud prawn show high levels of resilience against exploitation as the total population of this species remains relatively constant despite the removal of between 5000-48000 individuals per fortnight (Heinecken and Grindley 1982). Burrowing sand prawn *Callianassa kraussi* can be found in several sandy areas within the lower reaches, however this species is not as dominant as the mud prawn. Other biota which make up a significant proportion of the fauna of the Kowie Estuary include several crab species (*Sesarma catenata*, *Cyclograpsis punctatus* and *Scylla serrata*).

Estuaries are known to be important nursery habitats for many fish species, therefore it is not surprising that a large proportion of the ichthyofauna consists of juvenile marine species including various species of mullet (*Myxus capensis, Liza dumerilii* and *L. tricuspidens*), Cape stumpnose *R. holubi*, spotted grunter *Pomadasys commersonni*, white steenbras *Lithognathus lithognathus* and dusky kob *Argyrosomus japonicus* among others. Resident species include *Glossogobius callidus, Psammogobius knysnaensis, Gilchristella aestuaria* and *Hepsetia breviceps*. Whitfield *et al.* (1994) recorded 36 fish species in the Kowie Estuary, with Mugilidae and Sparidae being the most dominant families.

2.3 Transmitter-effect experiment

2.3.1 Study site and period

Prior to the acoustic telemetry study on *R. holubi*, a transmitter-effect experiment was conducted to determine whether the surgical implantation of acoustic transmitters into the species would have observable effects on the species. The transmitter-effect experiment was undertaken, from 13 June 2014 to 17 September 2014 (96 days), in the small-craft harbour within the Royal Alfred Marina in the Kowie Estuary (Figure 2.4). Juvenile *R. holubi* were caught at various sites along the Kowie Estuary using rod and line and kept in three independent holding cages, which were moored beneath a jetty. The specimens were kept in these holding cages for the duration of the experiment and were subjected to the ambient estuarine conditions.

Figure 2.4: Map showing the location of the Kowie Estuary in the Eastern Cape Province (A) and location of the experimental site in the Royal Alfred Marina illustrated by the red star (B).

Estuarine water temperature is often characterised by high variability due to solar radiation and seawater input. In particular seawater input during periods of coastal upwelling events can cause rapid short-term reductions in temperatures of up to 8-10 °C (Schumann and Pearce 1997). Water temperature was recorded hourly for the duration of the experiment by a temperature logger (Hobo® Water Temp Pro V2) moored alongside Receiver 2, approximately 610 m away from the cages. Water temperature ranged from 10.9 to 21.7 °C with a mean of 17.1 (± 1.2) °C (Figure 2.5). For the majority of the experiment, daily water temperature fluctuation was relatively low with the exception of three days (14, 17 June and 11 September 2014). On these days the difference between the minimum and maximum temperatures was 6.5, 10.3 and 5.9 °C, respectively (Figure 2.5).

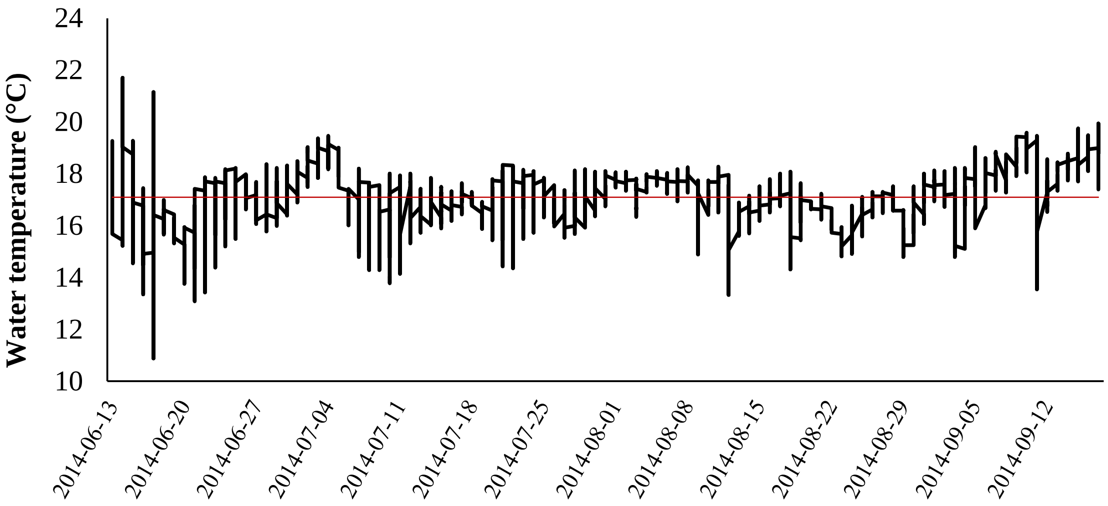

Figure 2.5: Water temperature (°C) recorded for the duration of the transmitter-effect experiment (13 June 2014 - 17 September 2014). The red line illustrates the mean water temperature for the entire experiment.

2.3.2 Research protocol

In total 42 juvenile *R. holubi* were used for the experiment with 14 fish assigned to one of three replicate holding cages (1 m^3) made of 9 mm oysternet mesh (Figure 2.6A). Within each of the holding cages the 14 fish were divided into two groups. One group of 7 individuals was surgically implanted with dummy transmitters (Figure 2.6B) made to the specifications of the Vemco V7-4L coded transmitters used in the field study (7 mm Ø, 1.6 g out of water), while the other group was kept as a control.

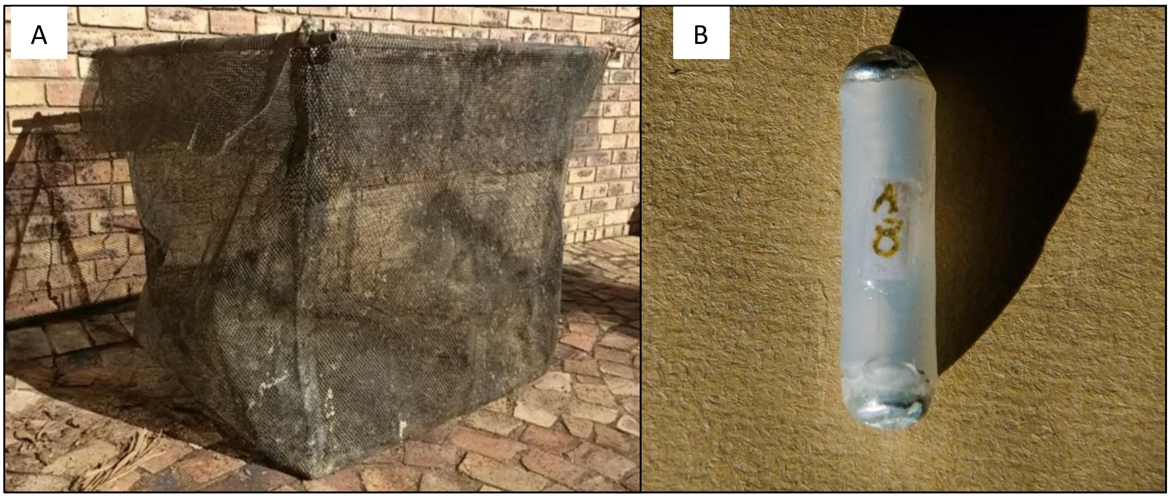

Figure 2.6: Examples of A) one of the holding cages and B) dummy transmitter used during the transmitter-effect experiment.

2.3.3 Surgical procedure

The control and treatment fish were anaesthetised using 2-phenoxyethanol (approximately 0.6 ml/L) in a 25-litre bucket of estuarine water. Once anaesthetised, each fish was measured and weighed to the nearest millimetre (fork length, FL) and gram, respectively. Each fish from the treatment group was then placed ventral side up on a damp cloth within a V-shaped cradle coated with high-density foam. An incision approximately 12 mm long was made in the ventral musculature in the region of the body cavity, through which a sterilised dummy tag was inserted into the body cavity. The incision was closed with two independent sutures (KRUUSE Suprimid sutures). Each fish was placed in a recovery bath of anaesthetic-free estuarine water. A fish was deemed to have recovered when it had resumed an upright swimming position. Once the fish had recovered, it was returned to its respective holding cage. The times of anaesthetisation, surgery and recovery were recorded.

2.3.4 Monitoring of fish

After the commencement of the experiment, each cage was checked once per day for the first three days for any mortalities. Thereafter, the cages were checked once per week for the next month and then once every two weeks for the remainder of the experiment. Three pilchards (*Sardinops sagax*), cut into small pieces, were provided to each cage twice per week. Each cage was also equally supplemented periodically with mudprawns (*Upogebia africana*) and white mussels (*Donax serra*). The fish were also observed feeding on the biofouling which grew on the side of the cages. The outside of the cages were periodically cleaned in order to reduce the amount of biofouling, which could have otherwise restricted water flow through the cages.

Upon completion of the experiment all the fish were sacrificed by submersion in ice water. The fish were then transported from the Royal Alfred Marina to the Department of Ichthyology and Fisheries Science general laboratory (Rhodes University, Grahamstown) where a health assessment of each fish took place. The health assessment consisted of an examination of the external appearance, internal organs, wound area and tag position. The health of each fish was ranked using several health metrics, including general appearance, fin erosion, wound inflammation and healing, stomach fullness, fat content, and liver and gill colour. The number of sutures present and the position of the dummy tag within the body cavity were also noted. Growth was assessed by comparing the overall differences in weight and length gain between the groups. Note that the mass of the dummy transmitter (1.6 g out of water) was subtracted from the final mass of each treatment fish so that the actual mass was used for all comparisons. The effect of transmitter implantation on recovery was assessed between groups by comparing the differences in post-anaesthesia recovery time.

2.4 Field telemetry experiment

2.4.1 Capture and tagging of fish

A total of 21 juvenile *R. holubi* was captured from a small boat using rod and line, and surgically implanted *in situ* with VEMCO V7-4L coded transmitters on 23 October 2014. These transmitters had an estimated battery life of 123 days and randomly transmitted signals (pings) every 40 to 80 seconds (i.e. nominal delay of 60 s) at a frequency of 69 kHz.

Based on the results of the transmitter-effect experiment (Chapter 3) the minimum size of the juvenile *R. holubi* that were tagged was 140 mm FL with the largest individual being 190 mm FL. Captured fish were kept in a 25-litre holding bucket of fresh estuary water until surgery. The surgical procedure was the same as described in the research approach for the transmitter-effect experiment. Once fish were deemed to have recovered they were released at their respective capture sites. Seven fish were captured, tagged and released at each of three sites along the estuary (upper ~18 km (-33°32'14.16", 26°48'0.25"), middle ~11.5 km (-33°33'26.68", 26°50'26.09") and lower ~5 km (-33°35'15.58", 26°51'53.56") from the mouth) (Figure 2.7). Tagging fish at three different sites was undertaken in order to alleviate any bias associated with highly resident individuals in a single area as well as to observe whether individuals from different sections of the estuary exhibited varying behaviour.

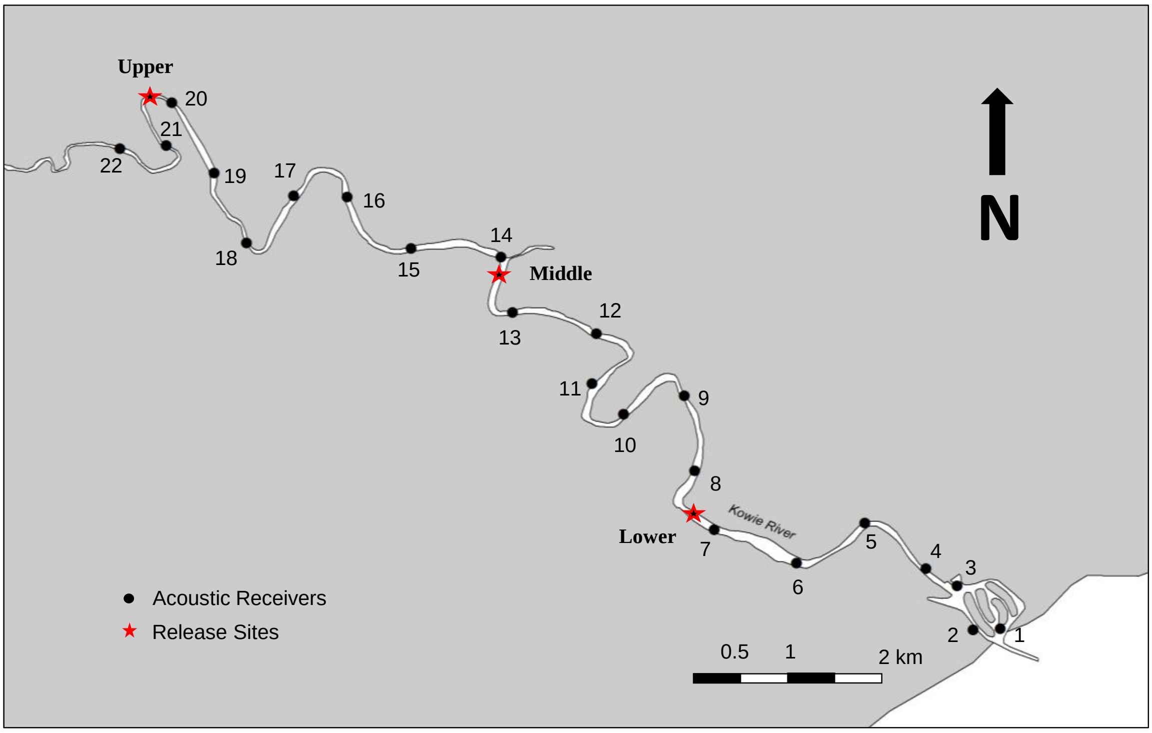

Figure 2.7: Map of the Kowie Estuary showing the position of the acoustic receivers (1 - 22) as well as the release sites (lower, middle and upper) of the tagged fish (red stars).

The size range of the juvenile *R. holubi* tagged in this studying was from 140 to 190 mm FL with a mean of 154.7 (± 14) mm (Table 2.2). There were no significant differences in fish size among the release sites (F = 1.19, p = 0.33; one-way ANOVA). The mean times to anaesthesia for the upper, middle and lower release sites were 92 (± 20) s, 66 (± 3) s and 82 (± 17) s, respectively (F = 4.96, p = 0.02; one-way ANOVA). A post hoc analysis (Tukey HSD test) showed a significant difference between times from the upper and middle release sites (MS = 236.68, df = 18, p = 0.015), but no significant differences between the upper and lower (MS = 236.68, df = 18, p = 0.45), and middle and lower release sites (MS = 236.68, df = 18, p = 0.17). The mean surgery time for all tagged fish was 178 (± 17) s with no significant differences among the release sites (F = 3.25, p = 0.06; one-way ANOVA). There were also no significant differences in recovery time among the release sites (F = 2.56, p = 0.11; one-way ANOVA) with the overall mean being 265 (± 146) s.

Table 2.2: Summary of tagging information of the 21 juvenile *Rhabdosargus holubi* tagged with V7-4L acoustic transmitters in the Kowie estuary on 23 October 2014.

Fish number	Release site	Fork length (mm)	Surgery duration (secs)
1	Upper Kowie	175	194
2	Upper Kowie	155	168
3	Upper Kowie	161	164
4	Upper Kowie	153	213
5	Upper Kowie	141	197
6	Upper Kowie	145	192
7	Upper Kowie	151	192
8	Middle Kowie	164	145
9	Middle Kowie	176	178
10	Middle Kowie	164	158
11	Middle Kowie	165	175
12	Middle Kowie	158	181
13	Middle Kowie	141	173
14	Middle Kowie	156	168
15	Lower Kowie	190	155
16	Lower Kowie	140	167
17	Lower Kowie	143	172
18	Lower Kowie	150	189
19	Lower Kowie	140	194
20	Lower Kowie	140	191
21	Lower Kowie	141	168

2.4.2 Monitoring of fish and data collection

An array of 22 stationery automated data-logging receivers (models VR2 and VR2W, VEMCO, Nova Scotia, Canada) moored approximately 1 km apart from each other along the length of the estuary (Figure 2.7), provided information on area use and movement patterns of individual tagged fish. These receivers identified and recorded the unique transmitter ID codes for each fish and stored the date and time of each detection. The receivers were strategically placed in areas of non-complex topography which allowed for adequate signal range (i.e. no 'shadowing' by solid obstacles) (Egli and Babcock 2004; Kerwath 2005). The receivers were first stationed at their respective sites prior to the capture and release of the tagged fish. Monitoring of the tagged fish commenced as soon as the individuals were released and continued until the battery power of transmitters had run out (28 February 2015). At the end of the field experiment (127 days after tagging), detection data from all receivers were uploaded to a portable computer.

2.4.3 Detection range testing and array efficiency

There is currently no standardised definition for detection range; however, the most commonly used definition is the maximum distance at which a transmitter can be detected by a receiver (Kessel *et al.* 2014). Detection range exists in passive telemetry because the sound waves emitted by the transmitters are subjected to 'spreading losses', refraction and attenuation as the sound waves move through the water column towards the receiver (Singh *et al.* 2009). Heupel *et al.* (2006) suggested that detection range is the most variable aspect of acoustic telemetry with a number of factors influencing the detection range. Factors include physical and chemical characteristics of the water body such as temperature, turbidity, salinity and suspended solids (Heupel *et al.* 2006; Topping and Szedlmayer 2011), surface conditions with regard to wind and wave action (Kerwath *et al.* 2007), water depth (Sakabe and Lyle 2010), substrate type and obstructions (Claisse *et al.* 2011), position of the receiver in the water column (Clements *et al.* 2005; Heupel *et al.* 2006), transmitter type (Simpfendorfer *et al.* 2008), ambient noise (Simpfendorfer *et al.* 2002; Payne *et al.* 2010b), water flow (Kerwath *et al.* 2009), and biofouling around the hydrophone of the receiver (Heupel *et al.* 2008). Knowledge of the transmitter detection range of receivers used in a passive telemetry study is fundamental to accurately describing the movement patterns and habitat utilisation of tagged fish (Kessel *et al.* 2014). Furthermore, a lack of understanding of the detection range of the receivers may lead to erroneous conclusions with regard to movement patterns, which could subsequently misinform management decisions (Payne *et al.* 2010b).

Numerous studies have recorded the detection range of the receivers used to track acoustically tagged fish. Næsje *et al.* (2012) recorded a detection range which varied from 110 to 610 m (Great Fish Estuary, South Africa), depending on the receiver position and the environmental conditions. Kerwath *et al.* (2005) found that maximum receiver detection range was 400 m (East Kleinemonde Estuary, South Africa). Detection ranges of 200 – 600 m were recorded in the Little Swanport Estuary (Tasmania) (Sakabe and Lyle 2010), 150 m in the Catalina Marine Science Center Marine Life Reserve (California, USA) (Topping *et al.* 2006), and 300 to 400 m in the Gippsland Lakes (Australia) (Hindell *et al.* 2008).

The maximum width of the Kowie Estuary is approximately 150 m. Therefore, based on the detection ranges of the above-mentioned studies, it was unlikely that an acoustically tagged *R. holubi* could have swum past a receiver without being detected. This was confirmed by the efficiency of the receiver array during the field telemetry study. Of the 21 fish monitored only two fish (6 and 11) moved past a receiver without being detected. The periods of non-detection, however, were not due to inadequacies of the receiver array or receiver efficiency, but rather represented anomalous events. Fish 6 was most likely preyed upon by a piscivorous fish (possibly *Lichia amia*) a few hours prior to the period of non-detection. There are two justifications for this hypothesis. Firstly, Fish 6 exhibited a sudden behavioural change from high residency in the upper reaches to a rapid movement downstream to Receiver 4 and back up to Receiver 21, which is similar to movements displayed by *Lichia amia*, (Figure 2.8, SAIAB unpubl. data). Secondly, after the probable predation event, the transmitter was detected for approximately two and a half days, after which it was not detected (last detection – 17 December 2014, 14:12:07). An explanation for this could be that after digesting the juvenile *R. holubi*, the predatory fish excreted the transmitter in an area out of reception range of any receiver. Fish 11 provides a more complex series of observations. This fish was relatively resident within the area around Receiver 14 for just over the first two months of the study. However, between 29 December and 31 December 2014 it moved from Receiver 14 to Receiver 1 without being detected. The most likely explanation for this is that an angler fishing in the vicinity of Receiver 14 caught Fish 11 and kept it as live bait to target larger predatory fish in the estuary mouth. Once the angler was in the estuary mouth area Fish 11 either escaped or was discarded and, hence, was detected by Receiver 1. Interestingly, Fish 11 proceeded to move back up the estuary and return to Receiver 14 where it remained until the transmitter battery was depleted.

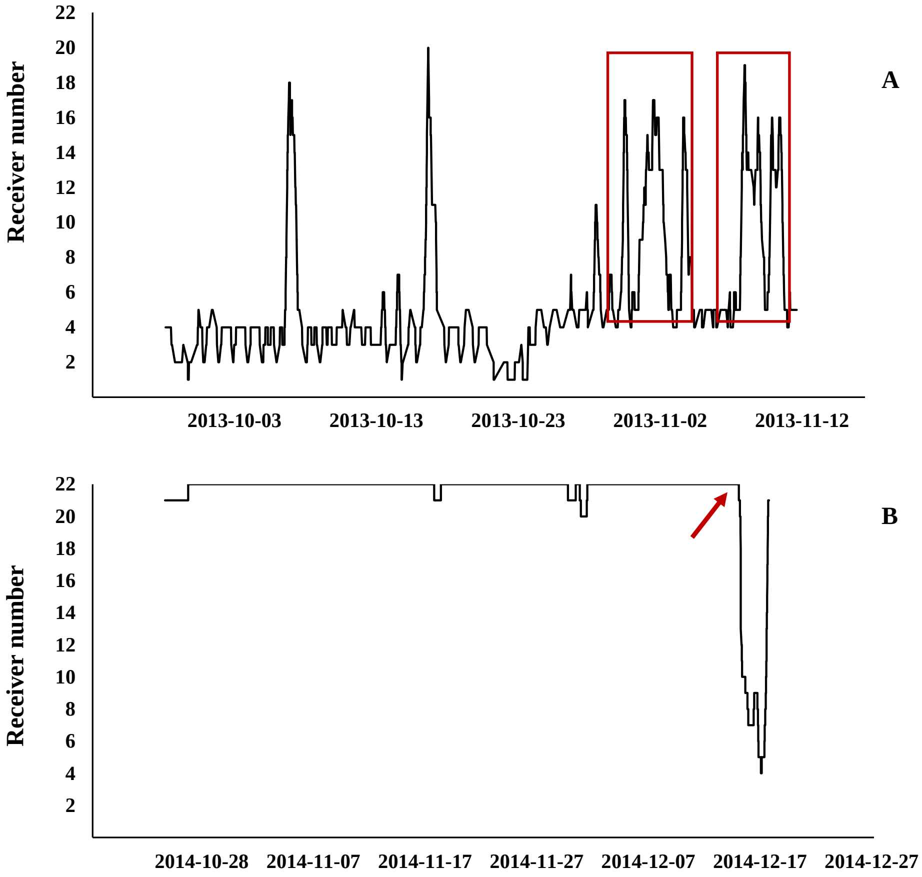

Figure 2.8: Comparison of A) typical individual movement pattern of Lichia amia (SAIAB, *unpubl. data*), and B) the individual movement of *R. holubi* (Fish 6) in the Kowie Estuary. The red blocks in graph A show periods of movement that are similar to that observed in graph B post the possible predation event (indicated by the red arrow) which matches the movement of *Lichia amia*.

Owing to the anomalous events experienced by Fish 6 and 11, the monitoring period used for data analysis for both fish lasted only until the anomalous event had occurred. This meant that the non-detection periods for both fish occurred after the events took place and did not fall within the monitoring period used in the study. Therefore, during the monitoring period for all the fish used in the telemetry study, the array efficiency was 100% with no fish moving past a receiver without being detected.

CHAPTER 3

EFFECTS OF SURGICALLY IMPLANTED ACOUSTIC TRANSMITTERS ON JUVENILE *RHABDOSARGUS HOLUBI*

3.1 Introduction

Acoustic telemetry has become one of the most popular and widely accepted techniques to study the movement behaviour and habitat utilisation of fish in their natural environment (Jepsen *et al.* 2005). A major reason is that acoustic telemetry eliminates the short-comings of conventional methods, such as mark and recapture, recreational fisheries surveys and commercial fishery logs, which only allow for discrete data and are limited with regard to quality and quantity of observations (Bridger and Booth 2003). Acoustic telemetry, on the other hand, allows for the continuous monitoring of an individual over a prolonged period in its natural environment (Bridger and Booth 2003). However, while acoustic telemetry is an effective tool to study the movement patterns of fishes, the reliability of the results is based on the assumption that tagged fish are not affected by the method of capture, surgical implantation and subsequent presence of the transmitter (Moore *et al.* 1990; Adams *et al.* 1998; Jepsen *et al.* 2002). Previous studies have shown that adverse effects on the physiology and behaviour of fish increase as the ratio of transmitter mass to fish mass increases (Marty and Summerfelt 1986; Greenstreet and Morgan 1989).

As a general rule, recommended by Winter (1983), the transmitter weight should not exceed 2% of the fish's weight out of the water. However, this rule has been challenged by a number of studies which have shown that exceeding the "2% rule" does not necessarily have adverse effects on the physiology and behaviour of the fish (Brown *et al.* 1999; Perry *et al.* 2001; Childs *et al.* 2011). Furthermore, Økland *et al.* (2003) showed that adverse effects may still occur even below the "2% rule". The discrepancy surrounding the "2% rule" suggests that the effects of transmitter implantation and presence vary with fish size and species (Bridger and Booth 2003). Therefore, the effects of transmitter implantation should be tested for each species specifically (Broadhurst *et al.* 2009; Butler *et al.* 2009), to ensure that there are no adverse effects on the physiology and behaviour of the subject (Winter 1983; Fabrizio and Pessutti 2007; Thorstad *et al.* 2009).

Several studies have suggested that dummy tag experiments should be undertaken prior to telemetry studies (Broadhurst *et al.* 2009; Ebner *et al.* 2009a; Thorstad *et al.* 2009), however few have done so. Jepsen *et al.* (2005) suggested that all telemetry studies should include some form of evaluation of tagging effects and that the effects being tested for should be relevant to the objectives of the study. Therefore, the overall aim was to test whether surgically implanting transmitters into juvenile *Rhabdosargus holubi* had a negative impact on their survival, growth and/or general health. The rationale behind this investigation was two-fold. Firstly, acoustic telemetry had not previously been applied to *R. holubi*, therefore information on how this species would respond to the surgical implantation and subsequent presence of an acoustic transmitter was unknown. Secondly, because of the small size of juvenile *R. holubi*, it was deemed necessary to undertake an experiment prior to a field telemetry study to determine whether the size and weight of the chosen transmitter was suitable, and the effects this might have on the fish. The specific objectives were to:

1) Determine the effects of surgically implanted dummy transmitters on growth, tag retention and mortality of juvenile *R. holubi*.
2) Assess the internal and external effects of the implanted transmitters on juvenile *R. holubi*.

3.2 Methods & materials

Details regarding the study site and research approach used for the transmitter-effect experiment are presented in Chapter 2.

3.2.1 Data analysis

Levene's tests showed homogeneity of variances for length and mass before (F = 1.77; p = 0.25 and F = 1.18; p = 0.34, respectively) and after (F = 1.5; p = 0.29 and F = 2.67; p = 0.18) the experiment. Shapiro-Wilk W tests showed that neither the length of the fish before and after the experiment (W = 0.94525, p = 0.04 and W = 0.93608, p = 0.02, respectively) nor the mass of the fish before and after the experiment (W = 0.88065, p = 0.0004 and W = 0.90376, p = 0.002) were normally distributed. Mann-Whitney U tests were, therefore, used to determine whether there were significant differences in the growth parameter values (mass and length) between treatment and control fish before and after the experiment. Mann-Whitney U tests were used to determine whether there were significant differences in the increase in growth parameter values (mass and length) between treatment and control fish over the experimental period.

In terms of time to anaesthesia, a Levene's test showed a lack of homogeneity of variances ($F = 8.22$; $p = 0.001$), therefore a Kruskal-Wallis test was used to test for differences among cages. A Mann Whitney U test was used to test for a difference in anaesthetic time between all the treatment and control fish. A Shapiro-Wilk W test showed that the recovery times of the fish were normally distributed ($W = 0.96$; $p = 0.12$). A one-way ANOVA was used to test for differences among recovery times between cages. Independent T-tests were used to test for differences in recovery time within all three cages. A Shapiro-Wilk W test showed that the surgery times of the fish were normally distributed ($W = 0.92$; $p = 0.089$). For surgical procedure duration, a one-way ANOVA was used to test for differences among the cages for the treatment fish.

A health assessment was conducted at the end of the 96-day experiment (mentioned in Chapter 2), which consisted of an internal and external examination. The methods used in the health assessment were based on those outlined by Childs *et al.* (2011). The health of each fish was ranked (highest number = good; lowest number = poor) using several health metrics. These included general appearance, fin erosion, wound inflammation, fat content, and liver and gill colour. The number of sutures present and the position of the dummy tag within the body cavity were noted. Additionally, the presence of parasites on the fish and the presence of cysts on the intestines of the fish were noted. Pearson's Chi Square tests were used to observe whether there were any differences between the control and treatment fish. Samuels *et al.* (2012) was used to select appropriate statistical tests.

3.3 Results

3.3.1 Mortality, tag retention and growth parameters

The 96-day experiment resulted in zero mortality, and 100% tag retention.

Mean length for the treatment fish was significantly greater than that of the control fish before the experiment ($U_{(40, 21)} = 142$; $Z = 1.96$; $p = 0.049$), but not significant after ($U_{(40, 21)} = 150$; $Z = 1.75$; $p = 0.078$) the experiment. There was no significant difference for the increase in length between the control and treatment fish over the course of the experiment ($U = 3$; $Z = 0.44$; $p = 0.66$) (Figure 3.1A).

Mean mass for the treatment fish before the experiment was significantly greater than that of the control fish (U $_{(40, 21)}$ = 140; Z = 2.01 p = 0.044). There was no significant difference between the mean masses of the treatment and control fish after the experiment (U $_{(40, 21)}$ = 148; Z = 1.81; p = 0.07). There was no significant difference for the increase in mass between the control and treatment fish over the course of the experiment (U = 3; Z = 0.44; p = 0.66) (Figure 3.1B).

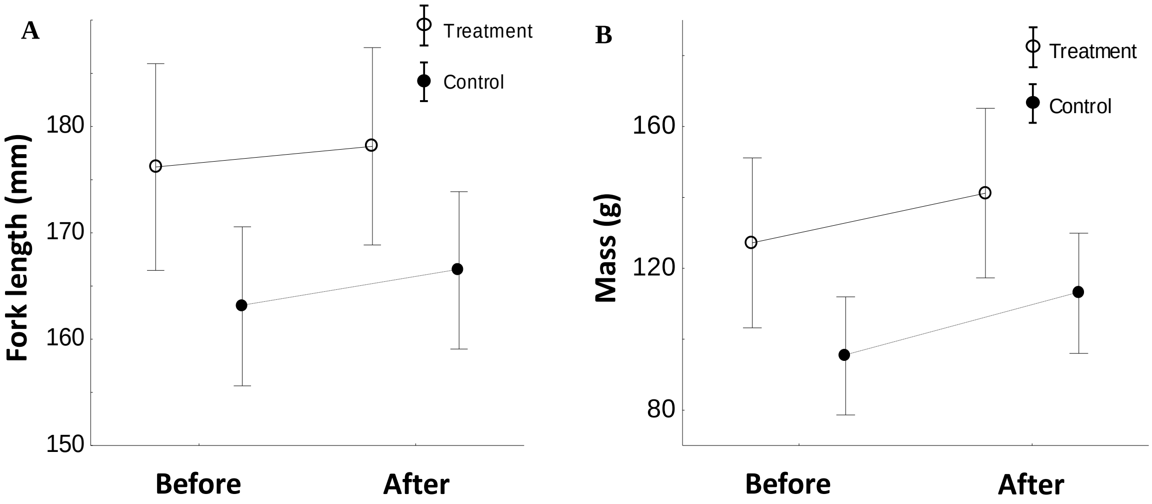

Figure 3.1: Comparison of mean growth parameters (± SD) before and after the 96-day transmitter-effect experiment. A) Change in fork length (mm); B) change in mass (g). Treatment refers to fish that were surgically implanted with dummy tags, while control refers to fish that were not.

3.3.2 Anaesthesia and surgery

Time to anaesthesia, surgical procedure time and recovery time were recorded for all fish. No significant differences were observed in time to anaesthesia among the three cages (H $_{(2, 42)}$ = 0.85; p = 0.66), however, the time to anaesthesia was significantly higher for the treatment fish (181 ± 57 s) compared to the control fish (119 ± 33 s) (U $_{(1, 40)}$ = 62; Z = -3.97; p < 0.0001). There was a significant difference among the cages in the length of recovery time (F $_{(2, 39)}$ = 10.2; p = 0.0003). Post hoc analysis (Tukey HSD test) showed that the recovery time of the fish in cage C (83.64 ± 44.67s) was significantly shorter than that for the fish in cages A (183.86 ± 59.14s; p = 0.0003) and B (148.43 ± 71.7s; p = 0.017).

Recovery time within cages were not different between the treatment and control fish for cage A ($t_{(1, 12)} = 0.2$; $p = 0.84$) and cage B ($t_{(1, 12)} = -2.13$; $p = 0.055$). However, within cage C the control fish (115 ± 39.97s) had a significantly longer recovery time compared to the treatment fish (52.29 ± 20.79s) ($t_{(1, 12)} = -3.68$; $p = 0.003$). There were no significant differences among the cages in the surgical procedure time for the treatment fish (201.57 ± 31.07s) ($F_{(2, 18)} = 0.09$; $p = 0.91$).

3.3.3 Health assessment

External examination after the experiment revealed that the general appearance of all fish was either 'good' or 'excellent' with no significant difference between the control and treatment fish ($\chi^2_{(1, 42)} = 0.93$; $p = 0.33$) (Table 3.1). This assessment revealed that 71% of the treatment fish and 57% of the control fish were in an excellent state, while the remaining fish were ranked as good. Four of the remaining six treatment fish were ranked as 'good', due to patches of red colouration either around the mouth or ventral region or both. The remaining control and treatment fish ranked as 'good' had slight fin erosion, but otherwise had no visible irregularities. Slight fin erosion was observed on 43% of the treatment and control fish (Table 3.1). Most of the fin erosion occurred on the caudal and anal fins, while only one control fish exhibited fin erosion on the dorsal fin. One treatment fish (ranked as 'good') had a damaged eye; however, this injury did not appear to have been inflicted during the experiment as it had healed relatively well. One treatment fish had slight wound inflammation around the area where the sutures penetrated the skin (Table 3.1). This particular fish was also the only individual which still had its sutures present. The remaining fish had expelled their sutures, with the wound areas having healed well, leaving only a slight scar.

Table 3.1: Health assessment of the 42 fish used during the 96-day laboratory experiment. Fish were ranked (highest rank number = good; lowest rank number = poor) using various 'health metrics'. Results are presented as the number of fish within each rank.

		Control (N = 21)	Treatment (N = 21)
External examination:			
General appearance	1 = Near dead	0	0
	2 = Bad	0	0
	3 = Average	0	0
	4 = Good	9	6
	5 = Excellent	12	15
Wound inflammation	1 = High inflammation	-	0
	2 = Slight inflammation	-	1
	3 = No inflammation	-	20
Fin erosion	1 = Extensive	0	0
	2 = Moderate	0	0
	3 = Slight	9	9
	4 = None	12	12
Internal examination:			
Fat content	1 = Low/none	8	12
	2 = Medium	13	9
	3 = High	0	0
Liver colour	1 = Orange	0	3
	2 = Red with orange patches	9	6
	3 = Red	12	12
Gill colour	1 = Pale pink	0	0
	2 = Pink	0	0
	3 = Red	21	21

The findings of the internal examination showed that the fat content was not significantly different between the control and treatment fish ($\chi^2_{(1, 42)} = 1.53$; $p = 0.22$), with 57% of the treatment fish and 38% of the control fish having little or no fat, and the remaining fish exhibiting a medium fat content (Table 3.1). Liver colour varied between red, red with orange patches, and orange with no significant differences observed between the treatment and control fish ($\chi^2_{(2, 42)} = 3.6$; $p = 0.17$). Completely red livers were observed in 57% of the control fish and 57% of the treatment fish (Table 3.1). The remaining control fish had livers with patches of orange varying in size. Livers with patches of orange were observed in 29% of the treatment fish, while the remaining 14% had livers which were completely orange. The gill colour of all fish was red (Table 3.1).

No parasites were observed and no cysts were observed on the intestines of any of the fish. The dummy tag of one of the treatment fish had moved dorsally within the body cavity, while the dummy tags in the rest of the treatment fish exhibited little to no movement. Only one of the dummy tags in the treatment fish was lying freely within the body cavity, while the remainder were either partially or totally encapsulated within a thin membranous layer.

3.4 Discussion

3.4.1 Mortality and tag retention

No mortalities were observed for either the treatment or control fish throughout the transmitter-effect experiment, therefore it is certain that the surgical procedure and subsequent presence of the dummy transmitters did not impede the survival of juvenile *R. holubi*. This is consistent with several studies which have shown that the implantation of dummy transmitters has little effect on survival. Attwood *et al.* (2007) observed the effects of VEMCO V8 transmitters (8mm Ø, 31mm long, 5.2 g mass) on 14 *Rhabdosargus globiceps* (276-334 mm FL). After 100 days, during which the fish were fed and inspected three times a week, no mortalities were reported. Thorstad *et al.* (2009) suggested that *Pomatomus saltatrix* are well suited for transmitter implantation because no tagging-related mortality was recorded. Childs *et al.* (2011) observed the effects of implanting two different sized transmitters in *Argyrosomus japonicus* over a 256-day experiment and found that no mortality could be directly linked to the presence of a transmitter. Adams *et al.* (1998) implanted radio transmitters into juvenile *Oncorhynchus tshawytscha* (114–159 mm FL) and reported that only one of 48 fish died. Although the above studies have reported no or low mortality rates, a few studies have experienced high mortality. For example, Morris *et al.* (2000), showed that *Coregonus nasus* experienced 50% mortality after surgical implantation, however this was in water temperatures above 16°C. Moreover, Økland *et al.* (2003) reported up to 100% mortality after surgically implanting transmitters into *Cyprinus carpio*. This discrepancy further highlights the need to test the effects of transmitter implantation on a species-specific basis.

A disadvantage of surgical implantation is the potential for loss or expulsion of the transmitter, which may occur through the incision area, body wall or via the intestine (Summerfelt and Mosier 1984; Chisholm and Hubert 1985; Lacroix *et al.* 2004). In this study, 100% transmitter retention was attained throughout the laboratory experiment. This is consistent with several other studies that have observed 100% tag retention in other species, for example *Centropristis striata* and *Paralichthys dentatus* (Fabrizio and Pessutti 2007), *A. japonicus* (Childs *et al.* 2011), *O. tshawytscha* (114–159 mm FL) (Adams *et al.* 1998) and *R. globiceps* (Attwood *et al.* 2007). In contrast, transmitter expulsion has been observed for a number of species including *Lepomis macrochirus* (Knights and Lasee 1996), *Ictalurus punctatus* (Summerfelt and Mosier 1984), *O. mykiss* (Lucas 1989), and juvenile *Salmo salar* (Moore *et al.* 1990; Lacroix *et al.* 2004). Factors that can increase transmitter retention rate include the selection of appropriately sized transmitters and the use of aseptic surgical procedures to reduce inflammation and adverse effects (Wagner and Cooke 2005). For long-term telemetry studies, transmitter retention must be ensured, and retention rates must be known prior to conducting the experiment (Fabrizio and Pessutti 2007).

3.4.2 Growth parameters

Growth is an important indicator of fish health (Wootton 1998). Transmitter implantation may affect the growth rate of a fish through physiological stress, physical injuries, increased susceptibility to diseases and parasites, altered social rank or other factors that may influence feeding or energy consumption (Thorstad *et al.* 2009). If growth is impeded by the surgical implantation of transmitters, fish behaviour may be altered or their susceptibility to mortality may increase (Brown *et al.* 2006). This would invalidate the assumption that a tagged fish may not be affected by the presence of a transmitter, and may impact the reliability of the results obtained from movement studies. Thus, quantifying the effects of transmitter implantation and presence on growth was necessary prior to undertaking the long-term field telemetry study.

Mean mass and length both increased at a similar rate for the control and treatment fish over the duration of the study, therefore surgical implantation and the presence of transmitters did not appear to adversely affect the growth parameters of juvenile *R. holubi*. A number of previous studies have also reported a lack of growth effects for several species, such as *A. japonicus* (Childs *et al.* 2011), *C. striata* (Fabrizio and Pessutti 2007) and *R. globiceps* (Attwood *et al.* 2007). Conversely, growth effects have been shown for species such as *P. dentatus* (Fabrizio and Pessutti 2007), *P. saltatrix* (Thorstad *et al.* 2009) and *O. tshawytscha* (Adams *et al.* 1998).

Over the duration of the experiment, the mean length increased by 3 mm and 2 mm for the control and treatment fish, respectively. Using a Von Bertalanffy model of the form $L_t = 358.1 (1 - e^{-0.24 (t - 0.77)})$ mm FL, which describes the growth of *R. holubi*, the mean length should have increased by 9 mm and 8.5 mm for the control and treatment fish, respectively (Farthing *et al.* in press). Moreover, Cowley and Whitfield (2002) using growth estimates from length-frequency data of *R. holubi* (50-150 mm) in the East Kleinemond Estuary, found that increase in length was 6.22 mm month^{-1}. Therefore, any treatment effect which may have occurred could have been masked by an overarching factor limiting the growth of all *R. holubi* used in the experiment. One particular factor may have been limited food availability, which would directly influence the growth of the fish. Fish in each holding cage were fed twice per week with a limited amount of food (not necessarily to satiation). Therefore, the amount of food ingested by each fish during the experiment may not have been adequate to maintain growth rates comparable to those of wild fish. Another factor which may have limited the growth rate was the season in which the experiment was undertaken. Blaber (1974) observed that the annual growth increment of juvenile *R. holubi* in the West Kleinemonde Estuary was approximately 60 mm, however growth was significantly reduced during winter when water temperature was low. In the present study, the majority of the experiment took place during winter months (June to September) when mean estuary temperatures were relatively low. The reduced growth observed could, therefore, be attributed to the cooler water temperatures during winter.

Growth parameters were significantly larger for the treatment fish compared to the control fish before the experiment. This was most likely due to sampling bias, with larger individuals subconsciously favoured for transmitter implantation over smaller individuals. By the end of the experiment, however, there was no significant difference between the growth parameters of the fish used in the experiment. This may be explained by the growth rates of different sized individuals. Smaller individuals generally grow at a faster rate than larger individuals of the same species, as is the case with *R. holubi* (Farthing *et al.* in press). It was, therefore, expected that the growth parameters of the individuals in this experiment would become similar over time.

3.4.3 Anaesthesia and surgery

Ebner (2009b) recommended that information such as time to induction, recovery from anaesthesia, and duration of surgery time should be reported for any telemetry experiment, particularly if the study species has not been previously tagged by surgical implantation. As surgical implantation of acoustic transmitters has not been undertaken in *R. holubi* prior to this study, the recording of the recommended information was necessary.

An ideal anaesthetic should swiftly induce a fish to anaesthesia, maintain it in a chosen state, and, when removed from the anaesthetic, allow for rapid recovery (Coyle *et al.* 2004). Generally, an increase in anaesthetic concentration will decrease the induction time (Hseu *et al.* 1998; Chambel *et al.* 2013; Mercy *et al.* 2014). Moreover, rapid induction time reduces recovery time (Coyle *et al.* 2004). Marking and Meyer (1985) suggested that the time to anaesthesia and recovery should not exceed 3 and 5 minutes, respectively. In the present study, the mean time to anaesthesia was significantly greater for the treatment fish compared to the control fish. As the treatment fish were generally larger than the control fish, this was probably related to the positive relationship between fish size and induction time (Coyle *et al.* 2004). The mean for the treatment fish (181.67 ± 57.67 s) slightly exceeded the 3-minute recommendation of Marking and Meyer (1985). However, the times for treatment fish were still within the range of other successful telemetry studies such as Fabrizio and Pessutti (2007), in which *C. striata* was induced in less than 5 minutes, and Ebner *et al.* (2009b) who induced complete anaesthesia of *Macquaria ambigua* in eleven to twelve minutes. Recovery time for all the fish (except one = 335 s) was below the 5-minute recommendation of Marking and Meyer (1985). The mean surgical time (201.57 ± 31.07 s) was within the recommended times of Cooke *et al.* (2003) (276.9 ± 7.8 s) and only slightly longer than that of Childs *et al.* (2011) (165 ± 37 s, range = 105 – 255 s).

The times recorded for all the facets of the surgical procedure (i.e. induction and recovery time, and surgery time) were within the recommendations of other successful studies. Therefore, if similar methods are replicated when surgically implanting transmitters into juvenile *R. holubi* during the field telemetry study, the data obtained should not be affected by the surgical procedure.

3.4.4 Health Assessment

The external and internal examination of the fish used during the laboratory experiment indicated that there was little difference in the general health between the control and treatment fish. Based on the health metrics used in the health assessment, most of the fish were either ranked as good or excellent illustrating the healthy condition of the fish and suitability of juvenile *R. holubi* as a telemetry species.

Only one treatment fish still had sutures present with the wound area slightly inflamed at the end of the experiment. According to Thorstad *et al.* (2009), the slight wound inflammation is not unexpected due to the ongoing process of suture shedding. The remaining fish, however, had shed their sutures by the end of the experiment with the wound area healing completely. Fat content ranged from 'low/none' to 'medium' for the control and treatment fish. This was expected owing to the possible limitation of food availability (already discussed). Adams *et al.* (1993) described a health assessment index (HAI) in order to evaluate general fish health. Two of the variables used in the HAI included gill and liver colour as a proxy for fish health. Therefore, gill and liver colour were used to assess the general health of *R. holubi* in this health assessment. The gills of all the fish in this study were healthy, while the majority of the fish maintained healthy livers (i.e. red). However, some of the livers were discoloured (either completely orange or had patches of orange). A number of reasons for liver abnormalities have been recognised, including bacterial and fungal infections (Wolke 1975), nutritional deficiencies (Snieszko 1972) and environmental pollutants (Couch 1975). Pale or discoloured livers are also commonly found in cultured fish fed with artificial diets (Tucker *et al.* 1997). Childs *et al.* (2011), while observing the effects of transmitter implantation on *A. japonicus*, found that all livers were discoloured and attributed this to the artificial feed used during the study. In the present study, even though there were slight differences, the majority of fish had similar liver colours, therefore transmitter presence seemed to have little effect on liver colour.

3.4.5 Conclusion and recommendations

The survival rate (100%) and tag retention rate (100%) observed during this experiment indicate that juvenile *R. holubi* (146-217 mm FL) is not adversely affected by the surgical implantation of acoustic transmitters (7 mm Ø, 1.6g out of water), and hence is well suited for acoustic tagging studies. The size and mass of the transmitters assessed were also suited to the size of *R. holubi* tested. The assessment of growth and health parameters, however, may have been affected by food availability during the experiment. It is therefore recommended that future experiments of a similar nature should ensure that all fish are fed daily to satiation.

It is assumed that, based on the assessment of the health and growth of the experimental fish, transmitter implantation would not have an effect on the movement of the fish used in the telemetry study. Therefore, provided that similar handling, anaesthesia and surgery techniques, and transmitter specifications are used, the outcomes of this experiment indicate that *R. holubi* is a suitable candidate for acoustic telemetry, and that a successful field study on juvenile *R. holubi* is possible.

CHAPTER 4

SPACE USE AND HABITAT CONNECTIVITY OF JUVENILE *RHABDOSARGUS HOLUBI*

4.1 Introduction

Estuaries play a crucial role in the life history of numerous coastal fishes around the world as they provide abundant food resources and protection from marine predators (Wallace and van der Elst 1975; Bennett and Branch 1990; Secor and Rooker 2005). However, although it is widely accepted that the offspring of many coastal spawning fishes recruit into estuaries (Whitfield 1990), there is a lack of information regarding their movements within estuaries. Moreover, few studies have observed the use of habitats adjacent to estuaries (i.e. marine and riverine), which may play a crucial role in the structure of marine fish populations (particularly species with distinct juvenile and adult habitats). Therefore, studying the space use patterns of juvenile fishes within the estuarine environment as well as the connectivity between adjacent habitats is fundamental to gaining a better understanding of fish ecology.

Space use and movement patterns have been found to be influenced by a number of factors, including an individual's size and reproductive mode (Dunlop *et al.* 2015), resource availability and habitat composition (Zeller 1997; Bennett *et al.* 2012), predator avoidance (Baldwin *et al.* 2002), and abiotic factors (Childs *et al.* 2008a; Payne *et al.* 2012; Gannon *et al.* 2015). Space use also varies among species and individuals within a species (Bacheler *et al.* 2009; Bennett *et al.* 2011; Dunlop *et al.* 2015).

Information on the exchange of individuals between different areas at various life history stages is essential to understanding population structure and dynamics (Vasconcelos *et al.* 2010), and recognising the importance of specific environments to a species (Able 2005). Connectivity for estuary-dependent species may be between the estuarine and riverine environments, or between the estuarine and marine environments. The use of the low-salinity riverine environment may be to reduce inter- and/or intraspecific competition for resources (James *et al.* 2008c), or for individuals to rid themselves of parasites (Hindell *et al.* 2008). Movement from the estuarine environment to the marine environment is suggested to be age dependent and is most often associated with an ontogenetic shift with the onset of sexual maturity (Whitfield 1990).

Acoustic telemetry allows for the constant monitoring of the movements of individual fish, making this technique a popular method to observe and quantify space use patterns and habitat utilisation within the estuarine environment. Estuarine movement patterns and habitat use of several southern African estuarine fish species have been determined using acoustic telemetry in the recent past, including dusky kob *Argyrosomus japonicus* (Cowley *et al.* 2008), white steenbras *Lithognathus lithognathus* (Bennett *et al.* 2012), spotted grunter *Pomadasys commersonnii* (Childs *et al.* 2008b) and leervis *Lichia amia* (SAIAB unpubl. data).

Rhabdosargus holubi is an abundant estuary-dependent species within many South African estuaries. The life history stages of this species are usually segregated with juveniles spending at least the first year of their lives within the estuarine environment and the adults within the marine environment (Whitfield 1998). Although much research has focused on the recruitment of post-flexion larvae into estuaries, little is known about their movement patterns within the estuarine environment or the use of adjacent environments.

This chapter aimed to assess the space use patterns and habitat utilisation of juvenile *R. holubi* in the Kowie Estuary using acoustic telemetry. Specific objectives included:

1) Identifying whether juvenile *R. holubi* exhibit a distinct movement behaviour (e.g. residency or ranging behaviour).
2) Determining whether individuals from different parts of the estuary display different movement patterns.
3) Quantifying the level of habitat connectivity and importance of riverine and marine environments to juvenile *R. holubi*.

4.2 Methods and materials

Acoustic telemetry was used to determine the space use patterns and habitat utilisation of *R. holubi* in the Kowie Estuary. A detailed description of the study site, the capture, tagging and monitoring of fish, as well as the data collection procedure can be found in the general methods and materials chapter (Chapter 2).

4.2.1 Data analysis

Several studies have raised concerns regarding the short term effects of transmitter implantation on fish behaviour and physiology. For example, Mulcahy (2003) suggested that fish implanted with transmitters may be reluctant to move after release and that this lack of movement may last from minutes to days. Furthermore, Kreiberg (2000) suggested that fish require up to 24 hours for physiological imbalances, such as calcium, magnesium and sodium blood concentrations, to equilibrate. Therefore, data acquisition should be delayed until the fish has become accustomed to the implanted transmitter (Rogers and White 2007). The transmitter-effect experiment undertaken in the present study suggested that the presence of a transmitter did not have adverse short-term physiological effects on juvenile *R. holubi*, therefore the first 24 hours of monitoring was excluded and data analysis commenced from the first detection after this period of time.

Space use in the estuary was quantified as the proportion of time spent at each receiver by each fish (Cowley *et al.* 2008). This was calculated as the sum of the time between two consecutive detections by a single receiver, and half the time between consecutive detections at adjacent receivers. The proportion of time spent at each receiver by each fish was used to determine the mean proportion of time spent at each receiver for all fish and by each batch of fish (i.e. upper, middle and lower).

The length of the estuary used by an individual was calculated as the distance between the upper- and lower-most receivers on which an individual was detected. Two fish (17 and 19) were detected by a single receiver (Receiver 7), therefore the estuarine length used by these fish was calculated as half the distance between Receivers 6 and 8. A Shapiro-Wilk W test was used to test for normality of the length of estuary used by the individuals showed that the data was not normally distributed ($W = 0.8849$, $p = 0.018$). The mean lengths of estuary used by the individuals from each batch were, therefore, compared using a Kruskal-Wallis test.

During this study some individuals were not detected for extended periods of time, having last been detected on either Receiver 1 or 22. Previous studies have categorised these periods as either trips to the sea or to the riverine environment, respectively (e.g. Cowley *et al.* 2008, Childs *et al*. 2015). In the present study, an individual was deemed to have made a trip to the sea or river if it had not been detected for 24 hours and had last been detected at Receiver 1 or 22 respectively. To determine habitat connectivity the proportion of time spent in each environment (i.e. sea, estuary or river) was calculated for each individual. The mean proportion of time was calculated for all fish, and for each batch separately. The proportion of time spent in each environment by all the individuals was compared using a Kruskal-Wallis test. The proportion of time spent in each environment by the lower, middle and upper batches of fish were compared using Kruskal-Wallis tests.

4.3 Results

The total number of detections per fish ranged from 822 (fish 7) to 106872 (fish 21) (mean = 17484.4 ± 23541.4), showing considerable variation among individuals (Table 4.1). Of the 21 fish monitored, 12 fish remained in the estuary for the entire study period (Table 4.1). The remaining fish either left the estuary (went to sea), were caught by an angler or were possibly eaten by a piscivorous bird or fish.

Table 4.1: The fork length, batch, number of receiver detections, and number of days monitored for each individual in the Kowie Estuary (* individuals possibly caught by an angler and not reported, or eaten by a piscivorous bird or fish; ** individuals which went to sea and did not return).

Fish #	FL (mm)	Batch	No. detections	Days monitored within estuary
1	175	Upper	4896	127
2	155	Upper	1793	11 **
3	161	Upper	11356	87 **
4	153	Upper	9561	63 **
5	141	Upper	13839	127
6	145	Upper	2835	43 *
7	151	Upper	822	127
8	164	Middle	8477	110 *
9	176	Middle	35049	127
10	164	Middle	2686	15 **
11	165	Middle	999	66 *
12	158	Middle	3750	65 *
13	141	Middle	6471	127
14	156	Middle	14943	126
15	190	Lower	32537	127
16	140	Lower	13053	61 **
17	140	Lower	15740	127
18	143	Lower	35298	127
19	141	Lower	11766	126
20	150	Lower	34430	127
21	140	Lower	106872	127

4.3.1 Space use patterns

Juvenile *R. holubi* exhibited high levels of residency, illustrated by the high proportions of time spent at Receivers 7, 14 and 20 corresponding to the lower, middle and upper batch tag and release sites respectively (Figure 4.1A). However, the proportion of time spent in the vicinity of the receivers at the three sites decreased from Receiver 7 (0.29 ± 0.45) to 14 (0.23 ± 0.38) to 20 (0.09 ± 0.19). When comparing each batch separately, the lower batch spent the largest proportion of time at a single receiver (7 = 0.85 ± 0.15) with the middle and upper batch decreasing, respectively (14 = 0.68 ± 0.32 and 20 = 0.23 ± 0.26, respectively) (Figure 4.1B).

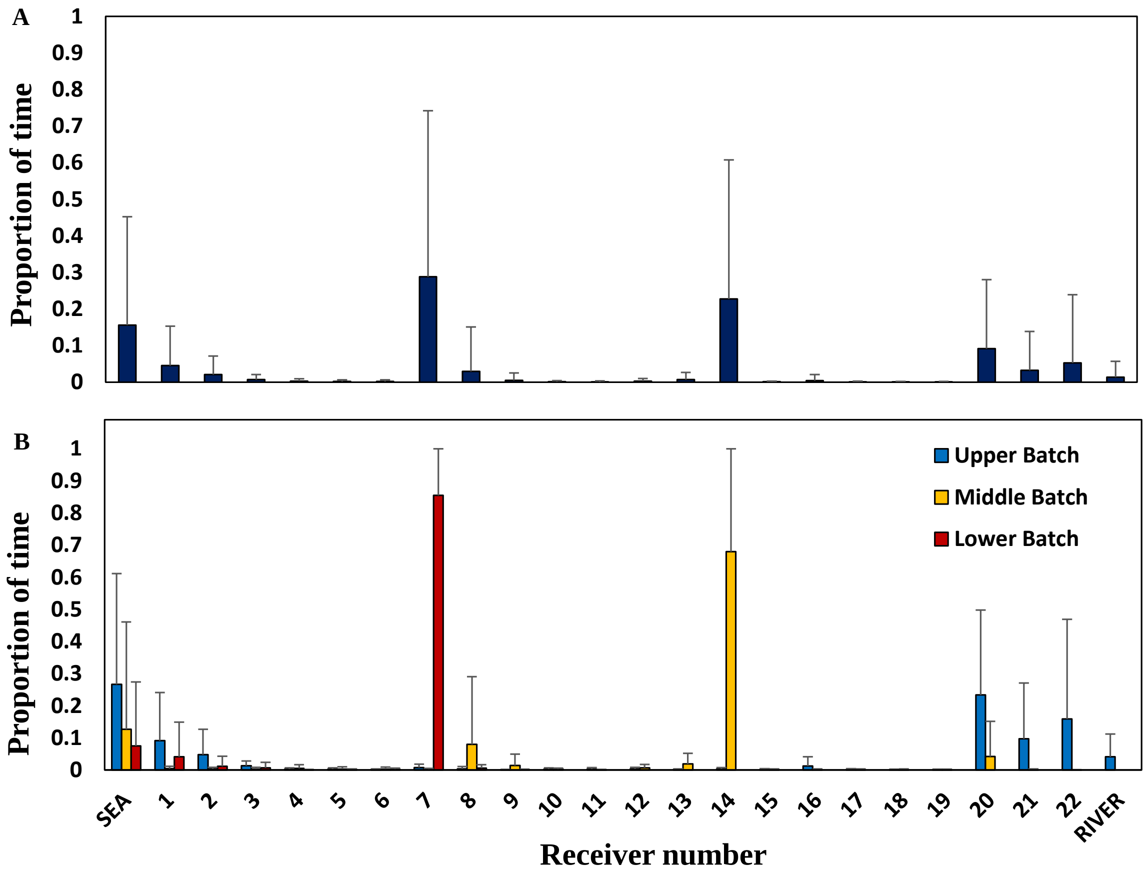

Figure 4.1: Mean proportion of time spent at each receiver by A) all fish, and B) fish from each batch. Vertical bars indicate upper standard deviation around the mean.

Space use by each individual was relatively similar within each batch (Figure 4.2). Six of the seven fish in the lower batch spent more than 95% (two of which spent 100%) of their time in the vicinity of Receiver 7. None of the fish in the lower batch ventured higher up the estuary than Receiver 9. Four of the seven fish from the middle batch spent at least 88% of their time in the vicinity of Receiver 14 (Figure 4.2). Two individuals (Fish 9 and 14) from the middle batch exhibited apparent home range shifts. Fish 9 moved upstream from the area around Receiver 14 to Receiver 20, where it spent 28% of its time. Fish 14 moved downstream from Receiver 14 to Receiver 8, where it spent 56% of its time. The individuals within the upper batch exhibited the lowest residency (Figure 4.2). Only one individual spent more than 80% of it time at a single receiver (Fish 6 – 81% at Receiver 22). Three of the seven fish from the upper batch were detected on all twenty two receivers.

One individual (Fish 11 – middle batch) was recaptured by a recreational angler on 6 November 2015 (more than 8 months after the study period had ended) within the vicinity of Receiver 14 (receiver closest to the tag site). Although its movements since the last detection are unknown, it is likely that this individual remained within the area around Receiver 14 for the majority of this period. Therefore, it is possible that this individual displayed long-term site fidelity.

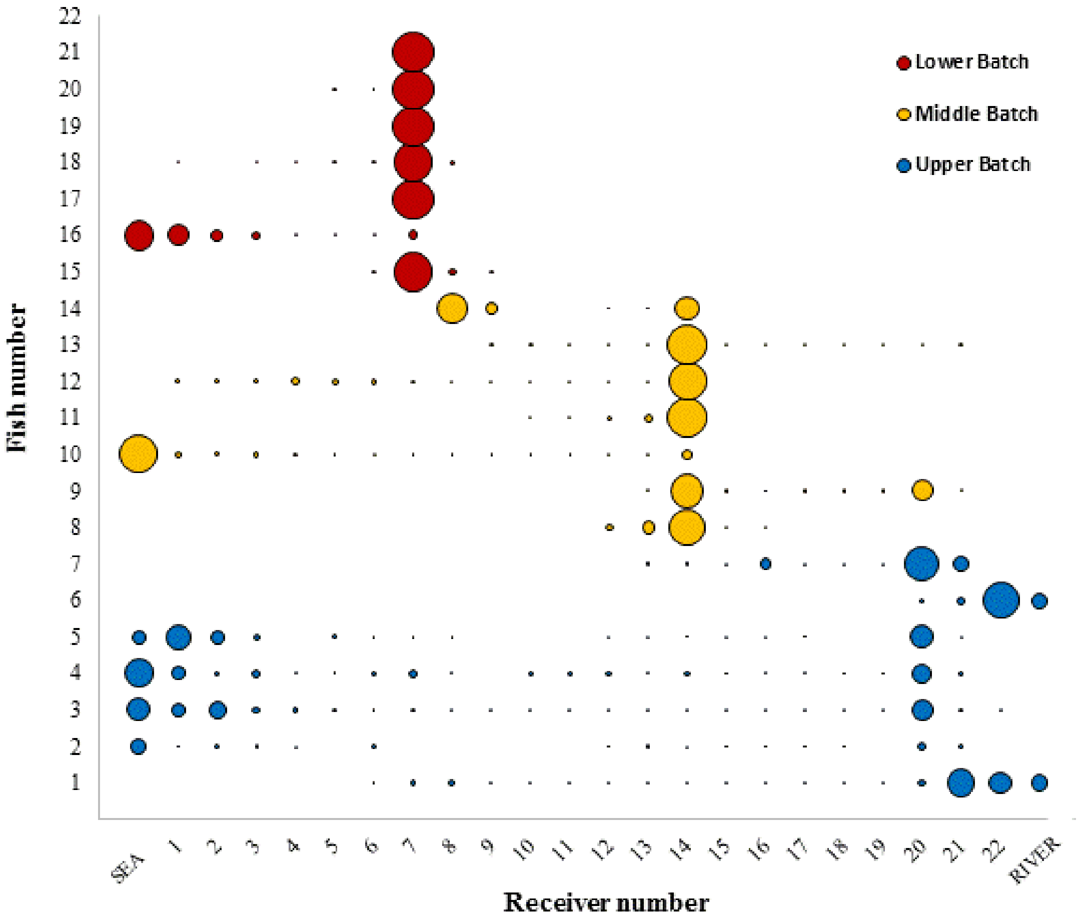

Figure 4.2: Bubble plot showing the proportion of time spent at each receiver for each individual fish.

The lengths of the estuary used by juvenile *R. holubi* from the upper and lower batches were significantly different ($H_{(2, 21)}$ =15.33; p = 0.0003) (Figure 4.3). The length of estuary used by the middle batch was not significantly different to that of the lower batch ($H_{(2, 21)}$ = 15.33; p = 0.17), or the upper batch ($H_{(2, 21)}$ =15.33; p = 0.14) (Figure 4.3).

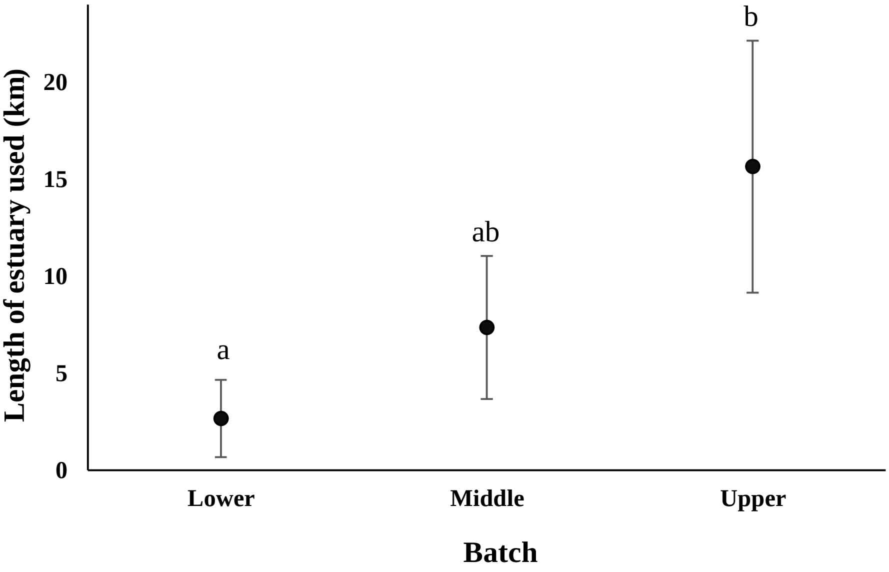

Figure 4.3: Length of estuary used by each batch of juvenile *Rhabdosargus holubi*. Batches with different letters (i.e. "a" and "b") indicate a significant difference in the length of the estuary used.

4.3.2 Multiple Habitat Use

Juvenile *R. holubi* spent the highest proportion of time in the estuarine environment (83%), with the sea (16%) and riverine (1%) environments being used to a lesser extent (Figure 4.4). The proportion of time spent in the estuary was significantly different to the sea and riverine environment ($H_{(2, 63)} = 43.59$; $p < 0.0001$). However, the proportions of time spent in the sea and riverine environment were not significantly different ($H_{(2, 63)} = 43.59$; $p = 0.986$). The lower, middle and upper batch spent 92%, 87% and 64% of their time in the estuarine environment, respectively. There was no significant difference between the times spent in the estuary by the upper and middle batch ($H_{(2, 21)} = 6.24$; $p = 0.14$), the upper and lower batch ($H_{(2, 21)} = 6.24$; $p = 0.13$), nor the middle and lower batch ($H_{(2, 21)} = 6.24$; $p = 1$). The lower, middle and upper batch spent 8%, 13% and 27% of their time in the sea environment, respectively (Figure 4.4). There was no significant difference between the times spent in the sea by the upper and middle batch ($H_{(2, 21)} = 3.16$; $p = 0.68$), the upper and lower batch ($H_{(2, 21)} = 3.16$; $p = 0.63$), nor the middle and lower batch ($H_{(2, 21)} = 3.16$; $p = 1$). The only batch to use the riverine environment was the upper batch (4% of their time) (Figure 4.4).

Six of the twenty one fish monitored in the study went to sea (Figure 4.2). Five of these went to sea and did not return to the estuary, while only one individual (Fish 5) made a sea trip and returned to the estuary after 10 days. Of the six fish that went to sea, four were from the upper batch and one from each of the middle and lower batches. Only two fish entered the riverine environment, both of which were from the upper batch (Figure 4.2).

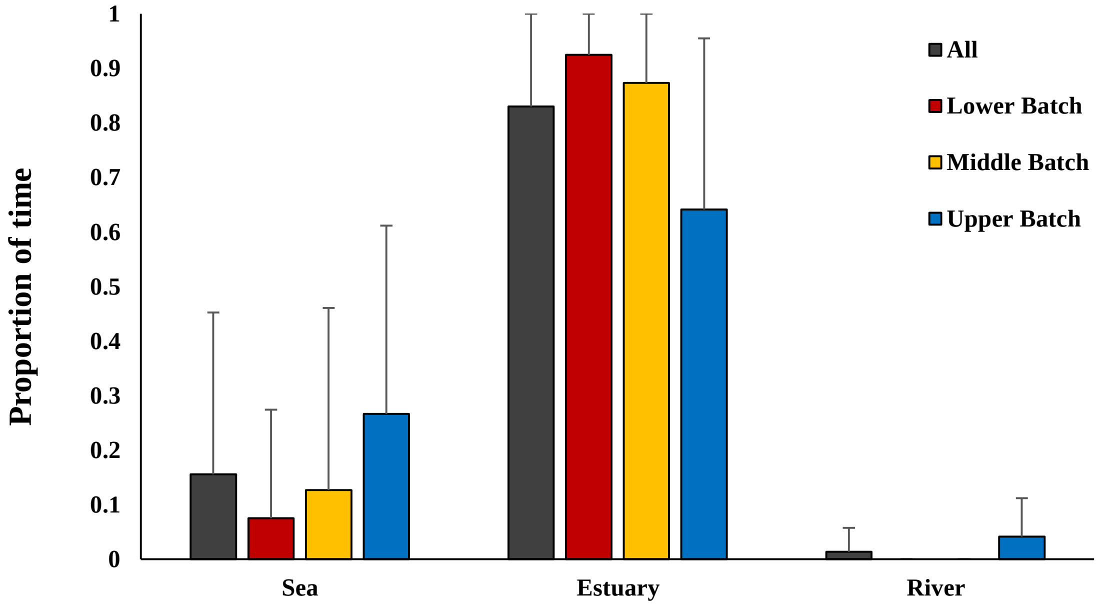

Figure 4.4: Proportions of time spent in the sea, estuarine and riverine environment by all juvenile *Rhabdosargus holubi*, and by each batch separately.

4.4 Discussion

4.4.1 Space use patterns

Juvenile *R. holubi* exhibited high levels of residency in the vicinity of their respective release sites. This was highlighted by the observation that 10 of the 21 fish tagged in this study spent more than 80% of their time within the area around a single receiver (< 1 km). Moreover, a further three individuals spent more than 80% of their time within the area of two receivers (approximately 2 km). Residency is common in a wide range of fish species, and within multiple habitats. Acoustically tagged California sheephead *Semicossyphus pulcher* in the Catalina Marine Science Center Marine Life Reserve (Santa Catalina Island, California, USA) spent the majority of their time in core areas as small as 600 m^2 (Topping *et al.* 2006).

Similarly, coral trout *Plectro leopardus* at Lizard Island (Great Barrier Reef, Australia) generally used home ranges up to 300 m^2 (Zeller 1997). Within the estuarine environment, juvenile white steenbras *L. lithognathus* (154–184 mm fork length) in the Great Fish Estuary (South Africa) also exhibited high residency within the mesohaline to oligohaline regions of the estuary and showed high levels of fidelity to their capture sites (Bennett *et al.* 2011). Furthermore, yellowfin bream *Acanthopagrus australis* showed high site fidelity in the St Georges Estuary (Australia) with core areas less than 500 m long (Gannon *et al.* 2015).

The long-term residency and high site fidelity displayed by juvenile *R. holubi* and numerous other species is not surprising as this behaviour is likely to have advantages over alternative behavioural strategies (Baras *et al.* 2002; Szedlmayer and Schroepfer 2005). Familiarity with an area may increase an individual's efficiency with regards to finding and accessing food resources, as well as utilising specific shelter sites, which may reduce the risk of predation (Zeller 1997). It is suggested that the relative abundance of submerged macrophytes (e.g. *Ruppia cirrhosa*, *R. maritima* and *Zostera capensis*) throughout the Kowie Estuary and the close association that juvenile *R. holubi* form with these plant species (Blaber 1973; De Wet and Marais 1990), mean that extensive movement in order to find food or to avoid predation may not be necessary, accounting for the high levels of residency observed. Other studies have observed similar resident behaviour in the presence of abundant resources. Eristhee and Oxenford (2001) suggested that *Kyphosus sectatrix,* an herbivorous species, preferred sites with greater algal cover and higher rugosity, which may have provided better feeding or shelter opportunities. Similarly, Childs *et al.* (2008c) suggested that juvenile *P. commersonnii* in the Great Fish Estuary (South Africa) made extensive use of areas where their preferred prey (*Upogebia africana*) was most abundant.

Although residency was high for all three batches, the degree of residency was highest for the lower batch and decreased for the middle and upper batches respectively, suggesting that the space use patterns of *R. holubi* were different among the batches. This was confirmed by the fact that the upper batch used a significantly greater length of the estuary compared to the lower batch. The relatively higher residency and fidelity to the release site displayed by the lower batch may be explained by the surrounding habitat. The presence of a large sandbank with extensive submerged macrophytes on the eastern side of the estuary may have provided an abundance of food resources and shelter from predation. Furthermore, the western shore is dominated by rocky substrate which drops off relatively steeply into the main channel of the estuary. The presence of this rocky substrate may provide additional shelter, particularly at low tide when the sandbank on the opposite shoreline has little water or is exposed. Matthews (1990) suggested that if a habitat is suitable and resources are available, a fish may stay within an area until the resources diminish. The habitat around the lower batch release site may have been favourable, hence the lack of extensive movement by juvenile *R. holubi*. In contrast, the food resources and shelter provided by submerged macrophytes may not have been as abundant in the area adjacent to the upper release site. For this reason, individuals may have been forced to move further in order to find adequate food resources or shelter.

The difference in space use among the batches may also be explained by the fact that individuals within the upper batch were farther away from the marine environment compared to the lower batch. They would, therefore, need to move a greater distance to facilitate the next ontogenetic phase of their lives (i.e. move to the sea to spawn).

The difference in movement behaviour exhibited by the different batches emphasises the importance of tagging fish along the length of an estuary. If all of the fish were tagged at a single release site (i.e. lower, middle or upper site), the findings of the space use patterns of juvenile *R. holubi* in this study would not be complete and the conclusions drawn from the results would not have been a true reflection of the entire population within the estuary. A number of studies have suggested it necessary and have attempted to tag fish throughout the study area in order to avoid the influence of location or habitat on movement patterns. Walsh *et al.* (2013) captured and tagged *Macquaria colonorum* at several sites in the Shoalhaven Estuary (Australia) and in different reaches of the estuary. Childs *et al.* (2015) tagged *A. japonicus* in the Sundays Estuary (South Africa) throughout the length of the estuary. Bennett *et al.* (2012) attempted to tag *L. lithognathus* throughout the East Kleinemonde Estuary (South Africa), but only managed to do so in the lower reaches due to the high fidelity of this species to the lower reaches of the estuary; a pattern that was subsequently shown to be consistent for a range of different estuaries (Bennett *et al.* 2015). Moreover, Sakabe and Lyle (2010) attempted to tag *Acanthopagrus butcheri* in the Little Swanport Estuary (Tasmania), but were only able to do so in the upper and middle reaches of the estuary and not the lower reaches. The high abundance and catchability of juvenile *R. holubi* in the present study allowed for the tagging of an equal number of individuals in three separate batches along the estuary within a single day. This allowed for the comparison of space use between the different batches.

4.4.2 Multiple habitat use

Habitat connectivity is a fundamental component of estuary-dependent species and studying the link between the different habitats used at different stages of a species' life cycle is crucial to understanding its ecology (Childs *et al.* 2015). Recently, habitat connectivity has received increased research attention with a number of studies suggesting that there is a paucity of information on this aspect of fish ecology (Gillanders 2002; Gillanders *et al.* 2003; Childs *et al.* 2015; Reddy *et al.* 2011).

Juvenile *R. holubi* spent most of their time in the estuarine environment. This is not surprising, as extensive research in estuaries has shown that *R. holubi* are abundant and ubiquitous in estuaries as juveniles, and are rarely caught in the sea (below 20 cm TL) (Blaber 1974; Heemstra and Heemstra 2004). The dependence on the estuary displayed by juvenile *R. holubi* in this study supports the findings of Carassou *et al.* (2016), who used stable isotope analysis to identify spatial and size-related variations in the diet of *R. holubi* in the Kowie Estuary, across three different environments (river, estuary and sea). These authors showed that different life history stages of *R. holubi* in the Kowie Estuary exhibited high site fidelity as the stable isotope analyses for juveniles residing in the estuary and lower part of the riverine environment reflected food resources from those particular sites.

Within southern Africa, numerous studies have shown the importance of the estuarine environment to the juvenile life stages of a number of fishery species, such as spotted grunter *P. commersonnii*, dusky kob *A. japonicus*, white steenbras *L. lithognathus* and leervis *L. amia* (Childs *et al.* 2008c; Cowley *et al.* 2008a; Bennett *et al*. 2011; Dunlop *et al.* 2015). An estuary-dependent species can be defined as one that would be adversely affected by the degradation or loss of the estuarine environment (Whitfield 1994a; Whitfield and Cowley 2010). Although juvenile *R. holubi* are known to use coastal rock pool habitats as nursery areas (Beckley 1985b), high levels of estuarine dependence observed during this study suggests that they would be adversely affected by estuarine degradation, and highlights the importance of estuaries as a nursery area for this species.

Although the upper reaches of the estuary were used extensively by juvenile *R. holubi*, the use of the riverine environment was limited to only two individuals (Fish 1 and 6) from the upper batch that ventured into this habitat. Fish 1 and 6 spent approximately 20 and seven days in the riverine environment, respectively. The use of the riverine environment by marine fish, particularly juveniles, is well documented for a number of species (Whitfield *et* *al.* 2003; Childs *et al.* 2008a; Cowley *et al.* 2008a; Hindell *et al.* 2008), however the degree of use varies among species. For example, freshwater mullet *Myxus capensis* spend the majority of their juvenile life stage in low salinity regions, probably to avoid competition with similar species (Whitfield and Paterson 2003; James *et al.* 2008c). The distribution of early juvenile *A. japonicus* (<150 mm) in the Great Fish Estuary (South Africa) was confined to the upper reaches (salinity 0 -5) probably due to predator avoidance, despite reduced availability of their favoured food item (*Mesopodopsis slabberi*) (Griffiths 1997). The use of the riverine environment by juvenile *R. holubi* may be due to a combination of factors. This species is an efficient osmoregulator (Blaber 1973), therefore the use of low salinity environments may reduce competition between other species that are not as well suited to such conditions, or such use may be a predator avoidance strategy. The use of the riverine environment may also have been to rid themselves of parasites that are unable to tolerate low salinities, as suggested by Hindell *et al.* (2008) in *Acanthopagrus butcheri*.

Six of the 21 individuals tagged in this study went to sea. With the exception of Fish 5, all these individuals did not return to the estuary once they had left. This supports the findings of previous studies that suggest that *R. holubi* undertake an ontogenetic habitat shift from the estuary to the marine environment with the onset of sexual maturity (Blaber 1973; Beckley 1983; Whitfield 1998). Several other species have also been shown to shift their habitat use with age. For example, spotted grunter *P. commersonnii* in the Great Fish Estuary made more frequent visits to the marine environment with increasing size, which may have reflected maturing of individuals (Childs *et al.* 2008b). Previous studies have suggested that *R. holubi* starts moving from the estuarine environment back to sea at a size of approximately 14 cm (FL) (Blaber 1973; Whitfield 1998). The fish tagged in this study ranged from 14 to 19 cm, therefore it is not surprising that some individuals entered the marine environment. The most probable reason for individuals moving into the marine environment was the imminent onset of sexual maturity, which is attained at approximately 19 cm (Heemstra and Heemstra 2004), with the spawning season occurring from July to February. The movement to the marine environment may also be due to an ontogenetic shift in diet. As individuals mature their dentition changes from sharp tricuspid juvenile teeth (suited to cropping plant material) to the adult's molariform teeth which are more robust and better suited to crushing shelled invertebrates in the marine environment (Blaber 1973).

4.4.3 Conclusions

This chapter emphasises the importance of estuaries as nursery areas to juvenile *R. holubi*. Although the riverine environment was used, it did not seem to be an important habitat for juveniles of this species. Within the estuarine environment, tagged individuals showed high levels of residency and fidelity to their capture and release site, however the degree of residency was dependent on the location of the release site with the upper and lower batch exhibiting different space use patterns. The fact that different space use patterns were observed among the batches emphasises the importance of tagging fish throughout the estuary. When juvenile *R. holubi* migrated to the marine environment they generally did not return, indicating an ontogenetic shift in habitat use with the onset of sexual maturity. In this chapter, biological factors were used to try explain the space use patterns displayed by juvenile *R. holubi*. However, environmental factors have also been shown to influence the space use and habitat utilisation of fishes.

CHAPTER 5

INFLUENCES OF GEOPHYSICAL CYCLES AND ENVIRONMENTAL VARIABLES ON THE MOVEMENT PATTERNS OF *RHABDOSARGUS HOLUBI*

5.1 Introduction

Estuaries are characterised by dramatic fluctuations in abiotic parameters, mainly due to the daily influx of saline water from the ocean as well as the inflow of freshwater from the land (Heupel and Simpfendorfer 2008; Walsh *et al.* 2013). Fish species that inhabit the estuarine environment have, therefore, evolved ways to cope with these fluctuations. Mobile species may move to areas of favourable conditions (Næsje *et al.* 2012), while the physiology of resident species allows individuals to tolerate wide ranges in abiotic parameters (Heupel and Simpfendorfer 2008). It is widely accepted that abiotic factors have a significant effect on the distribution and abundance of fish within estuaries (Blaber 1973; Szedlmayer and Able 1993; Whitfield 1994b, 1999). Therefore, determining the relationships between fish movement patterns and abiotic factors is essential to gaining a better understanding of the ecology of fish species in these systems (Childs *et al.* 2008a; Walsh *et al.* 2013).

Geophysical cycles (e.g. diel cycle, tidal cycle, lunar phase and season) are caused by the revolutions and orbits of the earth in relation to the sun, and the gravitational force exerted by the moon (Chabot and Watson 2010). These cycles can influence the movement and behaviour of organisms both individually and in combination. In terms of fish, particularly estuarine species, diel and tidal cycles have been recognised as important drivers of behaviour, which can vary among species. For example, certain species may be more active at night, when the low-light environment allows for protection from visual predators (Walsh *et al.* 2013), while other species are more active during the day as they rely on visual cues for foraging and predator avoidance (Dresser and Kneib 2007). Furthermore, some species move longitudinally along the estuary with the ebb and flow of the tide (Almeida 1996), while other species move horizontally onto submerged intertidal banks during the incoming tide and back into the deeper channel during the outgoing tide (Cartamil *et al.* 2003). Obtaining more information with regard to how individuals respond to these cyclical events will better our understanding of the factors which drive movement patterns of a species.

Habitat degradation of estuaries is a major threat to estuary-associated species, with some studies suggesting that it could lead to periodic or permanent elimination of certain species within estuaries (Cyrus 1991; Whitfield and Cowley 2010). Specific threats include *inter alia* water pollution, (urban and industrial runoff, and insecticides and herbicides from agriculture), canalisation of parts of the estuary, construction of dams in catchment areas, and excessive freshwater abstraction from rivers (Cyrus 1991; Whitfield and Cowley 2010). Such threats can cause large environmental fluctuations and adversely affect estuary-dependent species (Childs *et al.* 2008a). Therefore, knowledge of the responses of fish species to environmental variability may also increase our understanding of the potential effects of anthropogenic impacts on the estuarine environment.

Acoustic telemetry provides the ideal method to study the relationship between fish movement and abiotic factors, as real-time movements of individuals can be correlated with the corresponding ambient abiotic factors. This has allowed for unique insights with regard to the effects of abiotic factors on the movement behaviour of fish species.

The aim of this chapter was to investigate the influences of geophysical cycles (e.g. tidal and diel) and environmental factors (e.g. temperature, river inflow and barometric pressure) on space use patterns, estuary movements and habitat connectivity of juvenile *Rhabdosargus holubi*.

5.2 Methods and materials

Detailed descriptions of the study site, the capture, tagging and monitoring of fish, and the data collection procedure are presented in Chapter 2.

5.2.1 Influences of geophysical cycles on movement patterns

Cyclical movement patterns of juvenile *R. holubi* were identified using spectral analysis, based on the Fast Fourier Transform (FFT) method (Cooley and Tukey 1965). This method breaks down time series data into a finite sum of sine and cosine waves of different frequencies, which allows for the identification of cycles within the data (Hartill *et al.* 2003; Walsh *et al.* 2013). The results of this method are presented as a periodogram with Fourier amplitude on the y-axis and period on the x-axis. The upper limit of the period used on the x-axis of the periodograms was 30 hours, as this period would include both tidal and diel cycles. Peaks in Fourier amplitude indicate periods at which cyclical movement patterns have been identified. For example, if an individual exhibits a diel movement pattern, a peak in the Fourier amplitude would occur in the periodogram at 24 hours.

The acoustic detection data from each individual were subjected to the FFT analysis from 25 October 2014 at 00:00 (> 24 hours after the surgery) until the individual had left the estuary or the transmitter battery had expired. The receiver data for each fish were binned into 1-hour intervals, from which two metrics were calculated. Presence/ absence (PA) was calculated as whether or not an individual was detected by the receiver array during each 1-hour interval. Average receiver visited (ARV) was calculated as the weighted average receiver visited during each 1-hour interval. This value, calculated as the average of the receivers (receiver number) visited, weighed by the proportion of detections at each receiver within that 1-hour interval. For example, if an individual was detected once on Receiver 7 and twice on Receiver 8 within a 1-hour interval, the weighted average receiver would be 7.67. If an individual remained undetected during a 1-hour interval, the previous 1-hour interval ARV position was used. Presence/ absence and ARV were used as proxies for estuary movement.

An assumption when using the FFT approach is that the sample size of the data (number of 1-hour bins) is equal to a power of 2 (i.e. 2, 4, 8, 16, 32… 1024, 2048, etc.). Furthermore, the FFT is most effective when the sample size is greater than 1000 (Chatfield 2013). Therefore, fish monitored for less than 1024 hours were excluded from the analyses. The data series of the remaining fish were truncated at 1024 or 2048 hourly bins. In terms of PA data and as a result of the truncation and exclusion, 42.6 (1024 hourly intervals) and 85.3 (2048 hourly intervals) days of data were available for 5 and 14 individuals, respectively. In terms of ARV, individuals were also excluded if they were only detected by a single receiver for the entire study period; therefore 42.6 and 85.3 days of data were available for 5 and 12 individuals, respectively. Spectral (FFT) analysis was conducted in R software, version 3.2.1 (package: inbuilt in R; code author: Fred Menard) (R Development Core Team 2010).

5.2.2 Further investigation of cyclical movement patterns

The results of the FFT analysis identified two geophysical cycles that influenced the estuarine movement of juvenile *R. holubi*; namely diel and tidal. However, although FFT analysis shows the dominant cycles in an animal's movement, the analysis does not include a directional component (i.e. how the particular cycle affects the movement). Therefore, the effects of diel and tidal cycles on the movement of juvenile *R. holubi* required further investigation.

To determine how the diel cycle affected ARV, each day was divided into 24 1-hour bins (i.e. 1 = 00:00 – 01:00; 2 = 01:00 – 02:00; […]; 24 = 23:00 – 24:00) from 00:00 25 October 2014 until each individual was last detected. Within each 1-hour bin each day, the change in receiver visited was calculated as the difference between the highest and lowest receivers on which an individual was detected. For example, if an individual was detected by Receiver 3, 4 and 5 within an hourly bin, the change in receiver visited would equal two (i.e. 5 – 3 = 2). The average change in receiver visited was then calculated for each of the possible 24 hourly bins over the monitoring period of each individual. For example, if an individual was monitored for three days, it would have three 'change in receiver visited' values for each 1-hour bin (i.e. 00:00 – 01:00 = 3, 5, 1; average change in receiver = 3). The average change in receiver visited was plotted against daily hour.

Presence/ absence data were visually represented as contour plots, which were constructed in R. This allowed identification of whether an individual was detected more frequently during the night or during the day. Chi-square tests were run in R to determine whether there were significant differences in the number of detections for each individual between day and night throughout the study. "Day" was from sunrise rounded off to the nearest hour (e.g. 04:14 = 04:00, 05:55 = 06:00) until sunset, rounded off to the nearest hour. The remaining time was classified as "night". Sunrise ranged from 04:59 to 05:29 from 25 October 2014 to 27 January 2015, therefore "day" started at 05:00 during this period. Sunrise ranged from 05:30 to 05:59 for the remainder of the study period, therefore "day" started at 06:00 during this period. Sunset ranged from 18:32 to 19:22, therefore "night" started at 19:00 for the entire study period.

Tidal peaks were only observed in the spectral analysis of the PA data, and not for the ARV data; therefore the effect of tide was further explored in the PA data only. Furthermore, the influence of tide was only explored in those individuals exhibiting a tidal peak in the Fourier analysis. The method of determining how many times an individual was detected at either high or low tide was as follows. Firstly, the time of day of each low and high tide was rounded off to the nearest hour (e.g. 16:15 rounded off to 16:00, 16:45 rounded off to 17:00). The high and low tide periods were categorised as an hour before to an hour after the respective rounded off time (e.g. if high tide = 16:00, then high tide period = 15:00 to 17:00). The number of detections in each low and high tide period were recorded from 25 October 2014 until each individual was last detected (Table 4.1, Chapter 4). Chi-square tests were run in R to determine whether there were differences in the number of detections for each individual between high and low tide throughout the study.

5.2.3 Influences of environmental variables on movement patterns

General Linear Mixed Models (GLMMs) were used to statistically assess the influence of environmental variables on the movement patterns of juvenile *R. holubi.* These models were deemed appropriate as, unlike other statistical analyses, they are able to cope with non-independent data (inherent in telemetry studies) by incorporating certain variables as random effects (SPSS 2005; Kock *et al.* 2013). This allows for the modelling of spatially and temporally correlated data (Thiele and Markussen 2012).

The environmental variables tested in this study included rainfall (mm), barometric pressure (hPa), river inflow ($m^3.s^{-1}$), sea temperature and river temperature (°C). Rainfall and barometric pressure data, collected from the Port Alfred airport weather station (S33.5590; E26.8810), were obtained from the South African Weather Service (www.weathersa.co.za). River inflow data into the Kowie Estuary were obtained from the Department of Water Affairs (DWA 2015), at a hydrological station (P4H001) approximately 12 km upstream (S33.50678; E26.74444) from where the upper batch fish were released. Hourly sea and river temperatures were obtained from temperature loggers (Hobo® Water Temp Pro V2), which were moored alongside deployed acoustic receivers (2 and 22 respectively). Estuarine temperature data were also collected at Receiver 13; however, the data were highly correlated with river temperature and thus omitted from the analysis.

Environmental variables that were considered in the model building process included rainfall, rainfall with a 1-day lag (rainfall1), rainfall with a 2-day lag (rainfall2), log transformed rainfall (lograinfall), log transformed rainfall with a 1-day lag (lograinfall1), log transformed rainfall with a 2-day lag (lograinfall2), barometric pressure (bp), river inflow (inflow), river inflow with a 1-day lag (inflow1), river inflow with a 2-day lag (inflow2), sea temperature (seatemp) and river temperature (rivertemp). Certain variables were tested with a time lag as their influence may not have affected the individuals at the time of their fluctuation, but rather with a delay. For example, river inflow was recorded 12 km upstream from where the upper batch fish were tagged and released. Therefore it is likely that the effects of increased or decreased flow would have been delayed for a period of time. Daily rainfall is typically skewed due to the large proportion of zero/low rainfall days interspersed with sporadic high rainfall days. However, such skewness can be reduced by transforming the data (Hutchinson 1998). Rainfall data in this study were log transformed ($\log_{10}(x+1)$) in order to account for the large proportion of zero/low values.

The dependent/response variables used in the GLMMs were 1) daily position and 2) the daily length of estuary used. Daily position was calculated as the weighted average receiver visited during a single day. If an individual remained undetected for more than a day (24 hours), the previous daily position was used. Daily length of the estuary used was calculated as the distance between the upper- and lower-most receivers at which an individual was detected. If an individual was detected by a single receiver only, the length of estuary used was calculated as the sum of half the distances to each adjacent receiver.

The models firstly included all fish in the analysis to determine which variables had the greatest influence over a broad spatial scale. As all fish were included in these models, tagging batch (i.e. each group of fish - tagged at three different sites in the estuary) was included as a factor variable. In addition, GLLM's were run for each batch separately in order to determine whether the batches were influenced differently by environmental variables. For all the models, fish number (Fish ID) was assigned as a random effects variable.

The first part of the model building process involved running a number of models with various combinations of environmental variables (including all variables) against the dependent variables for all fish as a group and then for each batch separately. This was undertaken in order to determine which combination of variables would be used for the final model selection process. After 105 different variable combinations were run for each dependent variable, the environmental variables chosen for the final model building process included lograinfall, bp, inflow2, seatemp and rivertemp. An automated model selection process (AMSP) was performed in R using the "dredge ()" function in order to determine which model combination of environmental variables best fitted the data, whilst including batch as a factor and Fish ID as a random effect. In the AMSP, Akaike's Information Criterion (AIC) was used to compare the various models' goodness of fit to the data (Kadye and Booth 2014). The final models selected were those with the lowest AIC value. The relative strength of each model was based on the AIC delta value (Δ_i), which was calculated as the difference in AIC values between the best model and all other models. Models with $\Delta_i > 2$ have considerably less support (Burnham and Anderson 2002); therefore results of any model with $\Delta_i > 2$ were considered with caution. The AMSP was performed for both dependent variables for all fish and each batch separately (Note that batch was not included as a factor in the separate batch analyses).

After the final models had been run, the environmental variables that had significant relationships with the dependent variables were plotted against the 1) daily positions and 2) daily lengths of estuary used by each individual. This visual representation provided a better understanding of how the environmental variables influenced the individual movement patterns.

5.3 Results

5.3.1 Influences of geophysical cycles on movement patterns

Presence/ absence (PA) data for 19 of the 21 individuals monitored during this study were suitable for spectral (Fourier) analysis (Figure 5.1). Fish 2 and 10 were excluded from analysis as they were monitored for only 256 and 348 hours respectively. Nine of the 19 individuals (47%) showed diel peaks: three from the upper batch and six from the lower batch. Nine individuals showed tidal peaks: two from the upper batch, five from the middle batch, and two from the lower batch. Only three (16%) individuals (Fish 6, 17 and 19) showed both tidal and diel peaks in their PA data. Fish 6 and 19 showed primary diel peaks with secondary tidal peaks, whereas Fish 17 showed a primary tidal peak with a secondary diel peak. The remaining fish showed no clear trend and did not appear to be affected by any geophysical cycle.

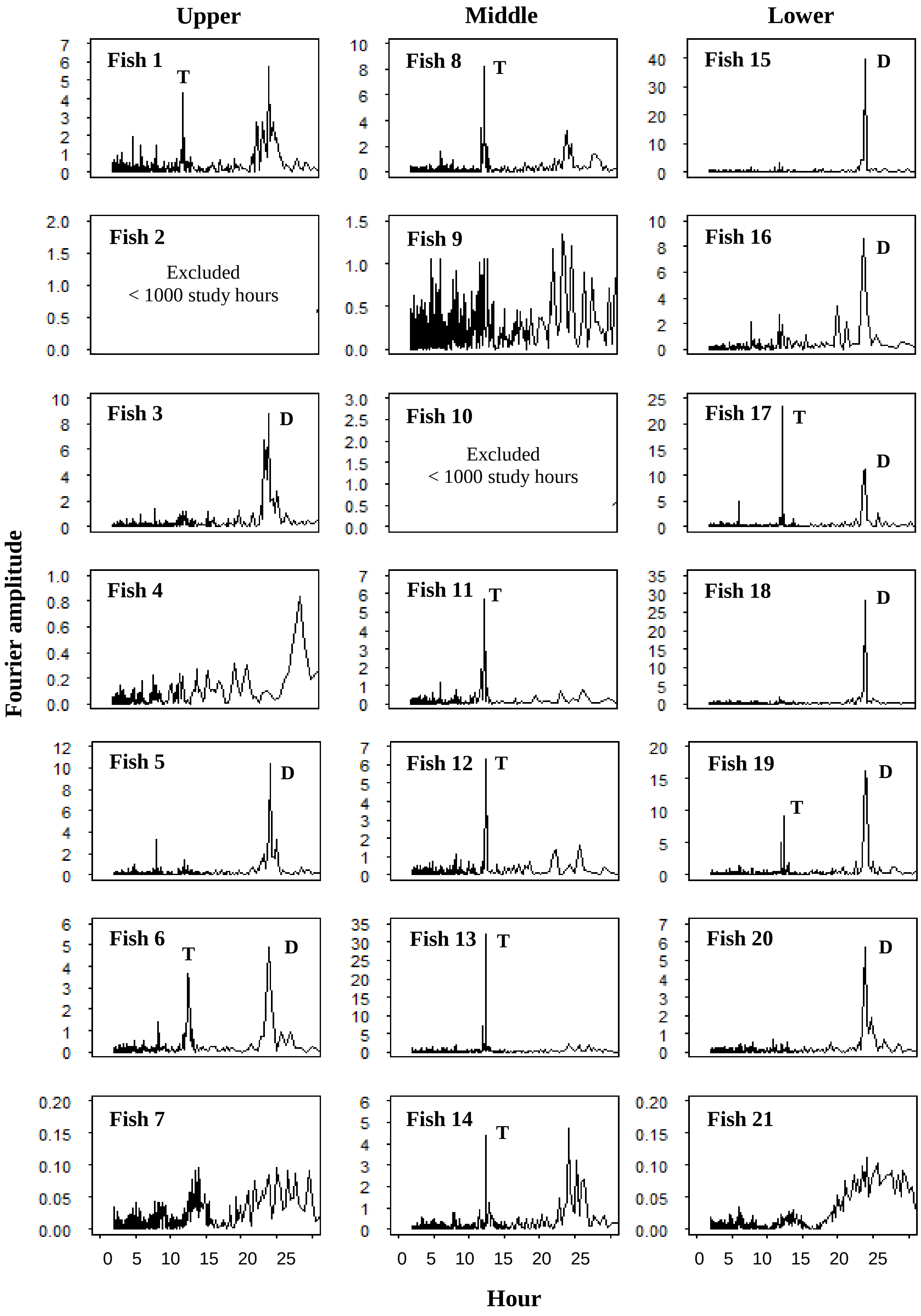

Figure 5.1: Periodograms of the spectral (Fourier) analyses of the Presence/Absence (PA) data for juvenile *Rhabdosargus holubi* from the Kowie Estuary (n = 21). The letter "D" indicates a peak over a diel cycle. The letter "T" indicates a peak over a tidal cycle.

The detection data of the nine fish that showed tidal peaks in the Fourier analyses (which only occurred in the PA data) were explored further. Six of these fish had significantly more detections at high tide, while the remaining three fish had significantly more detections at low tide (Chi-square tests, Table 5.1).

Table 5.1: Results of χ^2 tests on individuals that showed tidal peaks, showing which tide (High or Low) had significantly more detections. Degrees of freedom for all fish = 1.

Fish Number	Tide with more detections	χ^2 Value	P-Value
1	Low	42.604	< 0.0001
6	High	211.64	< 0.0001
8	Low	376.13	< 0.0001
11	High	7.4766	0.002
12	Low	40.699	< 0.0001
13	High	180.17	< 0.0001
14	High	140.41	< 0.0001
17	High	2192.6	< 0.0001
19	High	109.3	< 0.0001

Further investigation of the individuals that exhibited diel peaks showed that seven (Fish 3, 5, 15, 16, 17, 18 and 19) were detected significantly more frequently at night, while two (Fish 6 and 20) were detected more frequently during the day (Table 5.2 and Figure 5.2). Fish 20 showed two different patterns; one in whom it was detected more frequently during the day for the first 60 days, after which it was detected more frequently at night (Figure 5.2).

Two individuals (Fish 6 and 17) showed not only diel patterns, but also tidal patterns, represented by the areas of increased detections running parallel to each other from the bottom left towards the top right in the contour plots (Figure 5.2). These patterns suggest that either the time of day and the tidal phase influenced whether individuals were detected or not. Furthermore, the diel and tidal patterns on the contour plots supports the diel and tidal peaks shown in the Fourier analysis for these individuals (Figure 5.1). The periodogram for Fish 19 showed a tidal peak (Figure 5.1), however the contour plot did not show a clear tidal pattern compared to Fish 6 and 17 (Figure 5.2).

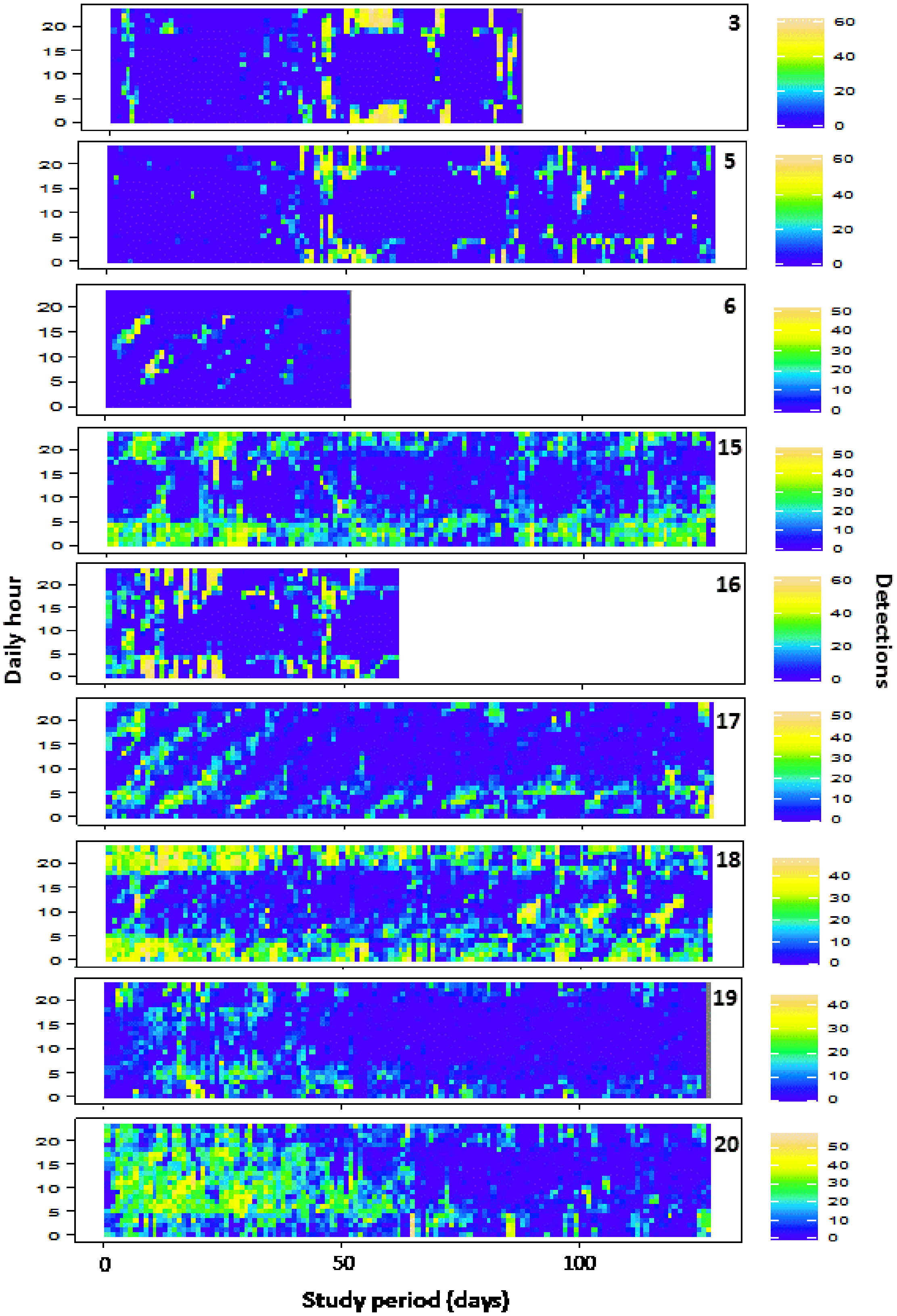

Figure 5.2: Contour plots for the hourly number of detections for the juvenile *Rhabdosargus holubi* that showed diel peaks in the spectral (Fourier) analysis of the presence/absence data. Blue areas show periods of low detection frequency; lighter colours show high detection frequency. Fish number is on the right hand side of each plot.

Table 5.2: Results of χ^2 tests on individuals that showed diel peaks, showing which time of day (Day or Night) had significantly more detections. Degrees of freedom for all fish = 1.

Fish Number	Period with more detections	χ^2 Value	P-Value
3	Night	2174.5	< 0.0001
5	Night	3112.4	< 0.0001
6	Day	1266.3	< 0.0001
15	Night	3449.5	< 0.0001
16	Night	758.3	< 0.0001
17	Night	11.1	0.0009
18	Night	4184.3	< 0.0001
19	Night	421	< 0.0001
20	Day	1648.7	< 0.0001

Seventeen of the 21 individuals monitored during this study provided data suitable for spectral analysis of ARV. Two individuals (Fish 2 and 10) were excluded because they were monitored for less than 1000 hourly bins. A further two individuals (Fish 17 and 19) were excluded because they were only detected by a single receiver for the duration of the study. Of the 17 fish analysed, two individuals (Fish 3 and 5) showed relatively distinct diel peaks (Figure 5.3). The remaining fish showed no apparent trend and did not appear to be affected by any geophysical cycle (Figure 5.3).

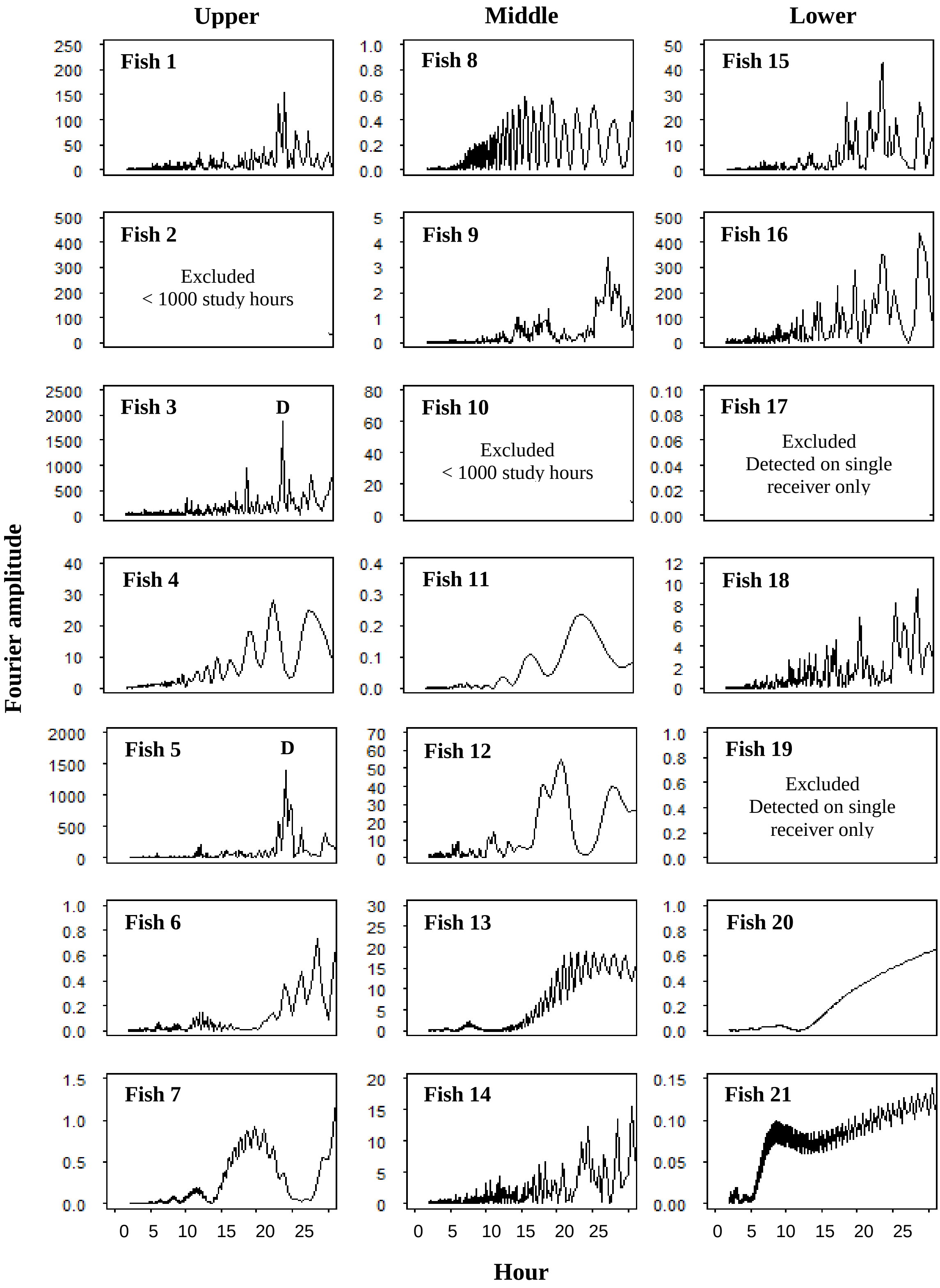

Figure 5.3: Periodograms of the spectral (Fourier) analyses of the average receiver visited (ARV) data for juvenile *Rhabdosargus holubi* from the Kowie Estuary (n = 21). The letter D (Fish 3 and 5) indicates a peak over a diel cycle.

Further investigation of the two individuals that showed diel peaks revealed that both made most of their movements during two periods of the day. Fish 3 made crepuscular movements up and down the estuary (i.e. changed position) between 03:00 and 09:00 and, to a lesser extent, between 15:00 and 21:00 (Figure 5.4). Average change in receiver visited decreased during the middle of the day and night. This individual did not visit more than a single receiver between 22:00 and 00:00 for the entire study period. Fish 5 made most of its movements early in the morning between 00:00 and 04:00 and again during the middle of the day between 11:00 and 17:00 (Figure 5.4).

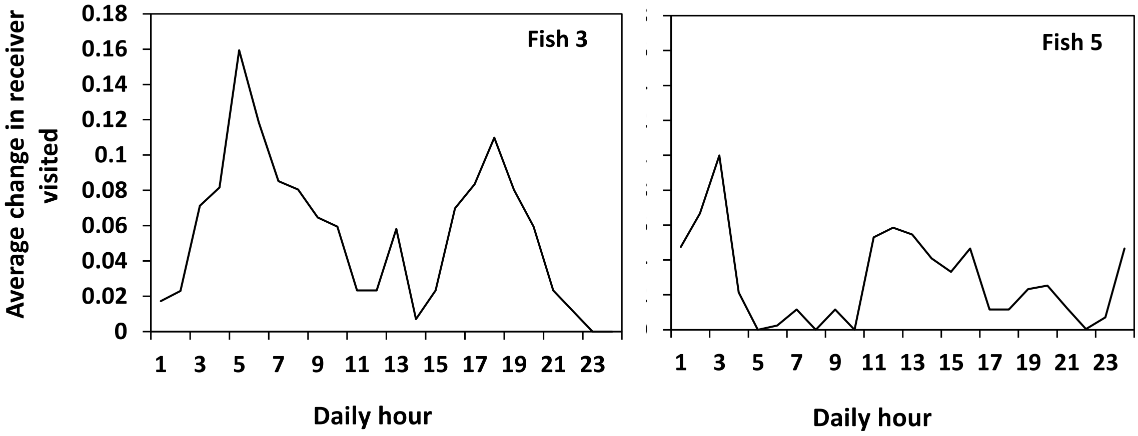

Figure 5.4: Change in average receiver visited during each daily hour for Fish 3 and 5.

5.3.2 Influences of environmental variables on movement patterns

The GLMMs fitted to the length of estuary used and daily position data varied in their appropriateness for explaining the movements of juvenile *R. holubi* based on the AIC and delta AIC (Δ_i) values (Table 5.3). Daily length of estuary used and average daily position models for the middle batch had delta values greater than 2, therefore these results were interpreted with caution. This was also the case for the average daily position model for the lower batch. The relatively high delta values for these models suggest that the environmental variables used may not have been appropriate for explaining the movement of the individuals from these batches and that other untested factors may have been more important with regard to influencing their movement patterns.

Table 5.3: Final models constructed for estimating the influence of environmental factors on the A) daily length of estuary used and B) daily position, for all individuals and for each batch separately, based on the automated model selection process (AMSP). FishID was included as a random factor in all of the models.

			AIC	**Delta AIC (Δ_i)**
A) Estuary Length Models:				
All fish	~	Inflow2 + Factor(Batch)	5480.3	1.22
Lower batch	~	Inflow2	978.7	1.28
Middle batch	~	LogRainfall	1725	3.00
Upper batch	~	Inflow2	2005.5	0.00
B) Daily Position Models:				
All fish	~	Inflow2 + River Temp + Factor(Batch)	11008.6	0.00
Lower batch	~	Sea Temp	1358.9	2.72
Middle batch	~	LogRainfall	2884.8	2.60
Upper batch	~	Sea Temp + Inflow2 + River Temp	3692.1	0.00

The GLMMs showed that a number of the environmental variables influenced the movement of individuals when all of the juvenile *R. holubi* were included (Table 5.4). Daily length of estuary used was significantly positively related to river inflow with a 2-day lag (W = 19.23, $p < 0.0001$) and batch as a factor (W = 6.25, p = 0.044) (Table 5.4). Average daily position was significantly negatively related with river inflow with a 2-day lag (W = 6.78, p = 0.009) and with river temperature (W = 172.37, p = < 0.0001), and significantly positively related with batch as a factor (W = 17.61, p = 0.0001) (Table 5.4).

Table 5.4: Summary statistics for the final models fitted to A) daily estuary length used and B) average daily position for all juvenile *Rhabdosargus holubi* (n = 21) monitored during the study. Factor(Batch)2 and Factor(Batch)3 = middle and upper batch respectively.

	Estimate	**S.E.**	**T-Value**
A) Model: Estuary Length ~ Inflow2 + Factor(Batch):			
(Intercept)	1.08535	0.13909	7.803
Inflow2	0.19549	0.04458	4.385
Factor(Batch)2	0.15540	0.19692	0.789
Factor(Batch)3	0.48561	0.19772	2.456
B) Model: Daily Position ~ River Temp + Inflow2 + Factor(Batch):			
(Intercept)	20.28664	1.80920	11.213
River Temp	-0.56399	0.04296	-13.129
Inflow2	-0.45051	0.17299	-2.604
Factor(Batch)2	6.14487	2.06883	2.970
Factor(Batch)3	8.38503	2.07036	4.050

The GLMMs showed that different environmental variables influenced the movement of individuals within the batches (Table 5.3). For the lower batch, the AMSP identified “Estuary length ~ Inflow2” and “Daily position ~ SeaTemp” as the most appropriate models (Table 5.3). The models indicated a significant positive relationship between daily length of estuary used and river inflow with a 2-day lag (W = 5.679, p = 0.017), and a significant negative relationship between average daily position and sea temperature (W = 7.081, p = 0.008) (Table 5.5 A).

For the middle batch, the AMSP identified “Estuary length ~ LogRainfall” and “Daily position ~ LogRainfall” as the most appropriate models (Table 5.3). However, the models indicated that there was neither a significant relationship between log rainfall and daily length of estuary used (W = 1.6045, p = 0.205) nor average daily position (W = 0.217, p = 0.642) (Table 5.5 B).

For the upper batch, the AMSP identified “Estuary length ~ Inflow2” and “Daily position ~ SeaTemp + Inflow2 + RiverTemp” as the most appropriate models (Table 5.3). The models indicated a significant positive relationship between daily length of estuary used and river inflow with a 2-day lag (W = 17.08, p < 0.0001) (Table 5.5 C). There were significant negative relationships between average daily position and sea temperature (W = 8.835, p = 0.003) and average daily position and river temperature (W = 132.617, p = < 0.001) (Table 5.5 C), but no significant relationship between average daily position and river inflow with a 2-day lag (W = 3.02, p = 0.082) (Table 5.5 C).

Table 5.5: Summary statistics for the final models fitted to daily estuary length used and daily average position for the A) lower, B) middle and C) upper batches of the juvenile *Rhabdosargus holubi* monitored during the study.

	Estimate	**S.E.**	**T-Value**
A) Models (Lower Batch)			
Estuary Length ~ Inflow2:			
(Intercept)	1.13841	0.11546	9.860
Inflow2	0.08117	0.03406	2.383
Daily Position ~ Sea Temp:			
(Intercept)	6.73463	0.68704	9.802
Sea Temp	-0.02195	0.00825	-2.661
B) Models (Middle Batch)			
Estuary Length ~ LogRainfall:			
(Intercept)	1.2855	0.1099	11.702
LogRainfall	0.1396	0.1102	1.267
Daily Position ~ LogRainfall:			
(Intercept)	12.7076	0.9894	12.844
LogRainfall	0.1252	0.2690	0.465
C) Models (Upper Batch)			
Estuary Length ~ Inflow2:			
(Intercept)	1.4386	0.1868	7.701
Inflow2	0.4802	0.1162	4.133
Daily Position ~ Sea Temp + Inflow2 + River Temp:			
(Intercept)	60.3751	3.6901	16.361
Sea Temp	-0.3350	0.1127	-2.972
Inflow 2	-0.8338	0.4795	-1.739
River Temp	-1.6395	0.1424	-11.516

River inflow with a 2-day lag was the only variable to significantly affect the daily length of estuary used. River inflow increased on four distinct occasions during the study (R1 – 17 November 2014; R2 – 26 November 2014; R3 – 7 December 2014; R4 – 6 February 2015) and during each of these occasions certain individuals exhibited an increase in the length of estuary used (Figure 5.5).

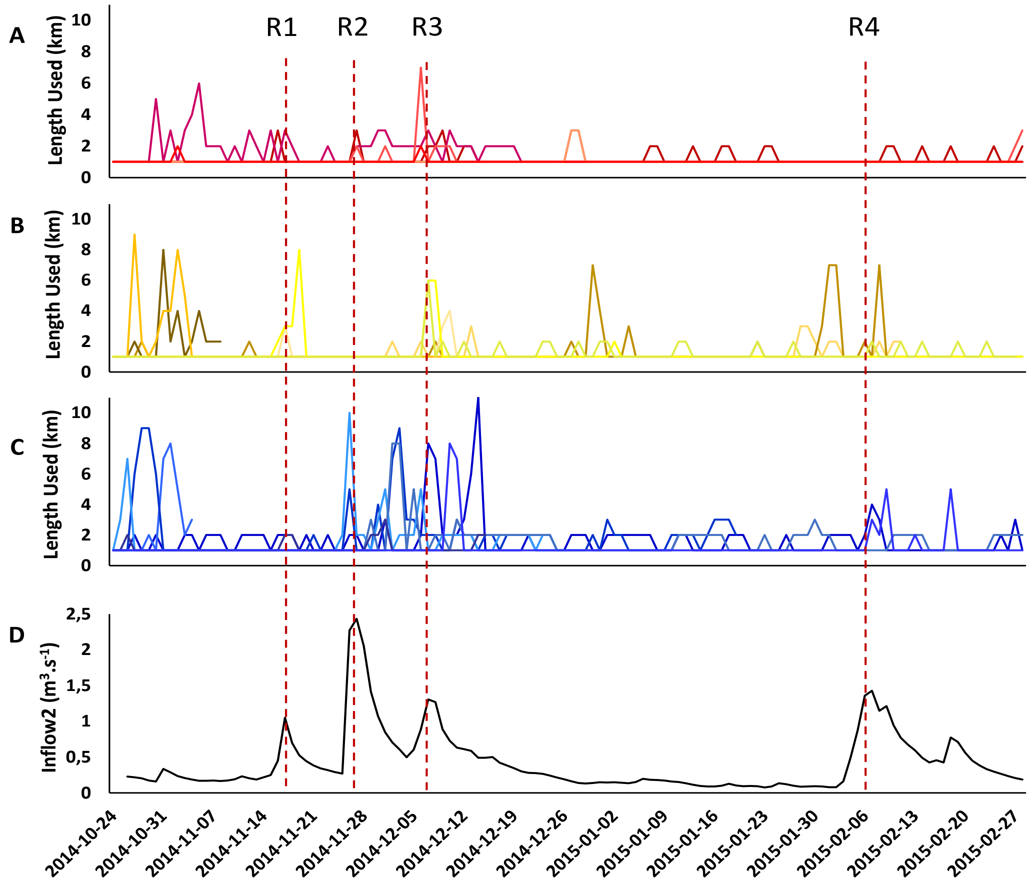

Figure 5.5: Daily length of estuary used by individual juvenile *Rhabdosargus holubi* tagged in the A) lower (n = 7), B) middle (n = 7) and C) upper batches (n = 7), plotted in relation to D) river inflow ($m^3.s^{-1}$) with a 2-day lag for the entire study period. Red dashed lines represent points in time where river inflow may have affected the daily estuarine length used by certain individuals. Different line colours within each batch (A,B and C) represent individual fish.

Average daily position was plotted against significant environmental variables (Figure 5.6). The red dashed lines (R1 – R3, Figure 5.6) show when river inflow appeared to influence the average daily position of certain individuals. At these points several individuals showed shifts in their average daily position. The first blue dashed line (B1, Figure 5.6) shows when (2 December 2014) river water temperature may have started to have a significant effect on the average daily position of certain individuals, particularly the upper batch. Before and after this point average sea temperatures were 16.6 (± 1.4) °C and 18.0 (± 2.0) °C, respectively, and river temperatures were 21.4 (± 1.1) °C and 25.5 (± 1.2) °C, respectively. The second blue dashed line (B2, Figure 5.6) shows a decline in sea temperature (2 December 2014) which may have forced an individual from the upper batch upstream.

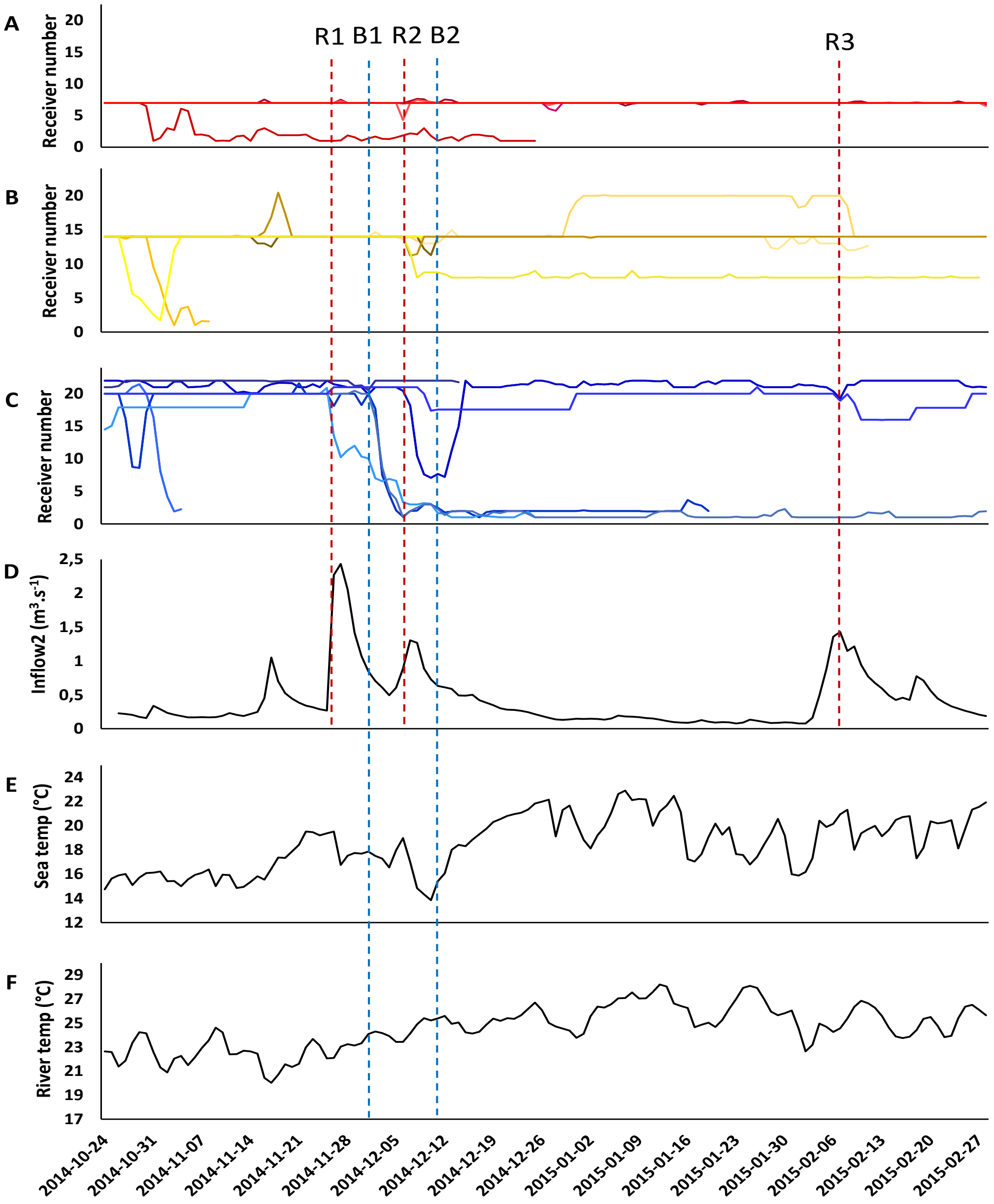

Figure 5.6: Daily average position of individual juvenile *Rhabdosargus holubi* tagged in the A) lower, B) middle and C) upper batch plotted against D) river inflow with a two day lag, E) sea temperature and F) river temperature for the entire study period. Red dashed lines represent points where river inflow may have affected the daily position of certain individuals. Blue dashed lines show where water temperature may have effected daily position.

5.4 Discussion

5.4.1 Influences of geophysical cycles on movement patterns

Spectral (Fourier) analysis was used to identify whether juvenile *R. holubi* in the Kowie Estuary exhibited rhythmic movement patterns driven by geophysical cycles. The analysis of the average receiver visited (ARV) data revealed that only Fish 3 and 5 displayed diel patterns with none of the other individuals displaying any distinct cyclical patterns. Both of these individuals displayed increased movement during two separate periods over the 24-hour daily cycle, however the timing of these increased movement periods contrasted. Fish 3 exhibited crepuscular movement behaviour, while Fish 5 displayed increased movement during the middle of the night and day. The opposite movement patterns of these two fish and the small sample of individuals which displayed diel peaks made it difficult to draw any conclusions using the ARV data. The lack of cyclical patterns for the remaining fish was most likely due to the high residency and fine-scale movements displayed by the majority of the individuals monitored during this study. Moreover, the use of ARV as a measure of movement was possibly not suitable as the spatial resolution of the receiver array may have been insufficient for the identification of such fine-scale longitudinal movements. In contrast, however, the presence/ absence (PA) data did reveal cyclical patterns for the majority of *R. holubi* tagged in the Kowie Estuary. Therefore, this section of the discussion will focus on the cyclical patterns observed in the PA data.

The spectral analyses conducted on the PA data showed that equal numbers of fish ($n = 9$) exhibited diel and tidal patterns, with only three individuals exhibiting both. These two cyclical patterns have been observed for several other species and are probably the most commonly observed behavioural patterns driven by geophysical cycles. For example, the main behavioural rhythms detected for *Pagrus auratus* in the Mahurangi Harbour Estuary, New Zealand, were diel and tidal (Hartill *et al.* 2003). Similarly, the dominant cyclical movement pattern exhibited by *Lithognathus lithognathus* in the Kariega and Sundays estuaries, South Africa, was diel, with tidal to a lesser extent (Bennett *et al.* 2015).

Further investigation of the individuals that displayed diel patterns showed that the majority were detected more frequently at night, which may be explained by the feeding ecology of juvenile *R. holubi*. Juvenile *R. holubi* generally feed during daylight with submerged macrophytes constituting the bulk of their diet (Blaber 1973; De Wet and Marais 1990). During daylight hours these fish may accordingly move to areas with increased submerged macrophytes, which could have negatively affected the acoustic receiver detection efficiency during the current study. Vegetation, among other physical barriers, has been shown to negatively affect detection efficiency (Baktoft *et al.* 2015). For example, detection efficiency of European catfish *Silurus glanis* in the Flix Reservoir, Spain, declined to zero near the littoral zone due to attenuation by vegetation (Carol *et al.* 2007).

Compounding the effect of submerged macrophytes is the shallow depth within which these plants occur. Several studies have suggested that detection efficiency is reduced in shallow environments. Claisse *et al.* (2011) showed that maximum detection range decreased with decreasing depth during range testing at Wawaloli (Hawaii). Furthermore, Sakabe and Lyle (2010) observed that detection range in the mouth area covered the deep entrance channel of the Little Swanport Estuary (Tasmania) but did not extend onto the adjacent shallow sand banks. In the present study, juvenile *R. holubi* may have used shallow and structurally complex, vegetated environments during daylight hours where detection efficiency was reduced.

Another possible reason for the greater prevalence of detections at night may have been that detection efficiency was reduced during the day due to environmental noise. Factors such as boating activity and increased wave action (caused by increased wind speed) during the day have been shown to reduce detection efficiency (Gjelland and Hedger 2013; Steel *et al.* 2014). In this study, however, the detection range testing results showed little difference in detection efficiency between night and day, therefore it is unlikely that individuals were detected more frequently at night for this reason.

Only two of the nine individuals which showed diel peaks were detected more frequently during the day. These individuals likely made use of structurally complex refuge areas at night, while moving to areas during the day where they were more likely to be detected. The increased detection frequency during the day may also be that these individuals occupied areas at night which were outside the detection range of the receiver array (i.e. between two receivers).

Six of the nine individuals that showed a tidal peak had significantly more detections at high tide, while the remaining three had significantly more detections at low tide. This suggests that, although tide did have an effect on these individuals, there was not an overarching movement pattern in response to tide. Hence, there may be a number of explanations as to why certain individuals were detected more frequently during high or low tide.

The location of the receivers within the estuary in combination with fine-scale fish movement patterns with the ebb and flow of the tide is a possible reason for the tidal peaks observed in the spectral (Fourier) analysis. Most of the juvenile *R. holubi* were detected by a single receiver for extended periods and generally did not move more than one kilometer (approximate distance between two adjacent receivers) during a tidal cycle. Presence or absence may have been due to tidal movements over short distances during which individuals would move in and out of the receiver detection range at either low or high tide. Furthermore, movements over short distances were probably the main reason for why tidal peaks were not observed in the spectral (Fourier) analysis of the ARV data. Hartill *et al.* (2003) showed that tidal movements of a similar sparid *P. auratus* in the Mahurangi Harbour Estuary (New Zealand) were only a few hundred meters long. In contrast, the sparid *A. butcheri*, is known to make frequent tidal movements of several kilometers with the current, in the Swanport Estuary (Australia) (Sakabe and Lyle 2010). Irrespective of the relative distances travelled, using the tidal flow may enable an individual to reduce the energy costs of movement, maintain its position within favourable environmental conditions, and/or increase its feeding efficiency by accessing favourable foraging habitats (Almeida 1996; Sakabe and Lyle 2010).

The higher detection frequency over high tide can be explained by the fact that higher detection efficiency has been shown to correlate with increased water depth (Claisse *et al.* 2011). Another explanation for the increased detection frequency during high tide may be that the individuals occupied areas outside of receiver detection range during low tide.

Individuals which were detected more frequently during low tide periods may have been using shallow areas (e.g. sand banks with submerged macrophytes) during high tide which were not within the detection range of a receiver. Bennett *et al.* (2015) found that this was the case for white steenbras *L. lithognathus* in the Kariega Estuary (South Africa) where individuals used shallow sandbanks during the high tide and the deeper channel during the low tide. Humston *et al.* (2005) showed that bonefish *Albula vulpes* in the northern Florida Keys moved onto shallow tidal flats during the high tide in order to access available food resources, and back to deeper channel areas during low tide. Sakabe and Lyle (2010) suggested that black bream *Acanthopagrus butcheri* may exhibit similar movement behaviour by utilising extensive tidal mudflats during high tide in the Swanport Estuary (Tasmania).

The presence of both tidal and diel peaks identified in the Fourier analyses, and parallel "lines" of increased detections on the contour plots (see Figure 5.2) highlight the influence of the two geophysical cycles on the movements of juvenile *R. holubi*. Other species which have been shown to be influenced by both diel and tidal cycles include *L. lithognathus* in the Kariega and Sundays Estuary (Bennett *et al.* 2015), *P. auratus* in the Mahurangi Harbour (Hartill *et al.* 2003), *A. butcheri* in the Little Swanport Estuary (Sakabe and Lyle 2010), and *M. colonorum* in the Shoalhaven River (Walsh *et al.* 2013).

Although the presence/ absence data showed that a number of individuals displayed diel and/or tidal peaks in the spectral (Fourier) analysis, the Fourier amplitude for the majority of these individuals was very low (maximum = 40), particularly when compared to other studies. Sakabe and Lyle (2010), who studied the movement patterns of black bream *A. butcheri* (Sparidae), presented periodograms with Fourier amplitudes ranging from 140 to 2500. While comparing patterns in acoustic detections from tagged cuttlefish *Sepia apama*, Payne *et al.* (2010) presented Fourier amplitudes which ranged from approximately 300 to almost 4000. Hartill *et al.* (2003) on the other hand presented periodograms with peaks that ranged between 13 and 50 for *P. auratus*, which is comparable to certain peaks identified in the movements of *R. holubi* during the present study. Although many of the diel and tidal peaks are relatively distinct, the low Fourier amplitudes suggest that some of these peaks should be interpreted with caution.

Understanding the movement patterns of juvenile *R. holubi* in response to geophysical cycles in this study was complicated by the low spatial resolution of the passive acoustic array relative to the scale of this species' movements (Hartill *et al.* 2003). Manual tracking provides higher spatial resolution data and the ability to track fine-scale movements of individual fish. Numerous studies have used a combination of manual and passive telemetry to track the movements of estuary-associated species including *inter alia P. commersonnii* (Childs *et al.* 2008b), *L. lithognathus* (Bennett *et al.* 2011), and *A. japonicus* (Cowley *et al.* 2008). The use of manual tracking in conjunction with passive tracking in this study would have allowed for a more detailed picture of the fine-scale movements of juvenile *R. holubi* and their relationship with geophysical cycles.

The results of this study do, however, contribute to our understanding of the relationships between the movement patterns of juvenile *R. holubi* and geophysical cycles. The absence of peaks in the ARV data show that individuals generally do not move extensive distances at different times of the day, nor during different tidal phases. Furthermore, the PA data suggested that movement is over relatively short distances (hundreds of meters) in and out of receiver detection ranges. These results further emphasise the high residency of juvenile *R. holubi*.

5.4.2 Influences of environmental variables on movement patterns

The high residency and site fidelity exhibited by juvenile *R. holubi*, in spite of fluctuating environmental conditions during the study, support previous findings that this species is able to tolerate wide ranges in environmental variables (Blaber 1973). This lack of extensive movement relative to the resolution of the deployed acoustic array meant that changes in fish distribution in relation to subtle variations in environmental variables were difficult to detect. Several environmental variables did, however, have a significant influence on both the length of the estuary used and the positions of individuals within the estuary. Furthermore, not only did specific variables have overarching influences on all tagged individuals (i.e. all batches combined), certain variables also influenced each batch separately, and variably.

Water temperature

Most fish (including *R. holubi*) are poikilothermic, meaning that their body temperature is dependent on the ambient water temperature (Fry 1971). Water temperature is thus regarded as one of the primary factors influencing the activity and behaviour of fish (Hanson *et al.* 2008). Water temperature also affects a number of life history characteristics including metabolism, growth and spawning (Hanson *et al.* 2008). Generally, fish have an optimum thermal range and will often alter their behaviour in order to maintain their position within these conditions (Childs *et al.* 2008a). This may involve moving horizontally from warmer shallower water near the littoral zone to cooler deeper water in the channel (or vice versa) (Whitfield 1999), or moving along the length of an estuary in order to find favourable conditions (Childs *et al.* 2008a).

Modelling of the effects of environmental variables on *R. holubi* behaviour showed that water temperature had a significant effect on their distribution within the estuary. River temperature had a significant negative relationship with daily position (i.e. movement towards the mouth with an increase in river temperature), when all individuals were grouped, and for the upper batch separately. The movement of individuals towards the mouth, particularly by the upper batch, may be explained by the relationship between water temperature and salinity tolerance of *R. holubi*. Blaber (1973) showed that between temperatures of 12 and 26 °C the interaction between water temperature and salinity was minimal. However, as temperature increased above 26 °C and salinity decreased below 35, *R. holubi* tolerance of these conditions was reduced. During the present study, river temperatures ranged from 19.0 to 29.7 °C with the mean temperature generally increasing over the study period. Therefore, it is possible that the increased river temperature together with the low salinities experienced in the upper reaches, forced certain individuals to move towards the mouth in order to maintain a position within favourable conditions.

Sea temperature did not significantly affect the daily position of juvenile *R. holubi* overall, and had no effect on the middle batch separately. However, sea temperature did have a significant negative relationship with the lower and upper batch (i.e. movement towards the mouth with an increase in sea temperature or vice versa). It is not surprising that sea temperature affected the lower batch as these individuals remained in relatively close proximity to the mouth region for the majority of the study. Sea temperature was recorded at Receiver 2, approximately 17 km from where the upper batch was tagged and released, therefore it is unlikely that sea temperature was a major driver of daily position for the individuals that remained within this region. However, sea temperature could have influenced the individuals which had already moved downstream from the upper reaches towards the mouth. These individuals may have been forced back up the estuary when sea temperature decreased, resulting in the negative relationship between daily position and sea temperature. For the individuals that remained in the upper reaches, river temperature was more likely the main driver of the movement towards the lower reaches of the estuary, as a change in temperature in this portion of the estuary could directly influence their physiology and behaviour.

Numerous studies have shown that water temperature has an effect on the distribution of fish in estuaries. DeCelles and Cadrin (2010) observed that most departures of winter flounder *Pseudopleuronectes americanus* out of the Plymouth Estuary (United Kingdom) occurred when water temperature was above 12 °C. Childs *et al.* (2008a) found that there was a higher abundance of spotted grunter *Pomadasys commersonnii* in the upper region of the Great Fish Estuary when sea temperature dropped below 16 °C, and suggested that these fish were making use of the warmer water temperatures within this region as a refuge. Matich and Heithaus (2012) showed that almost all juvenile bull sharks *Carcharhinus leucas* left the Shark River Estuary (USA) after an extreme decline in water temperature. Collins *et al.* (2008) showed that cownose rays *Rhinoptera bonasus* left the Caloosahatchee Estuary (USA) when water temperature dropped below 10°C.

The significance of water temperature on juvenile *R. holubi* in this study may also lead to seasonal changes in the distributions of individuals. During summer, water temperatures in the upper reaches generally increase, while the increased frequency of coastal upwelling events cause sporadic decreases in water temperature in the lower reaches (Singh *et al.* 2009). This may cause individuals from both the upper and lower reaches to move towards the middle reaches increasing competition, reducing habitat availability, and increasing susceptibility to exploitation in this region. As water temperature decreases in the upper reaches and upwelling events become less frequent during winter, the distribution of individuals may become more widely spread throughout the estuary. Future studies could focus on the relationship between seasonal change in water temperature and movement patterns and distribution by using transmitters which are able to monitor individuals for a full seasonal cycle.

River inflow and rainfall

River inflow (with a 2-day lag) had a significant positive relationship with the daily length of estuary used and a significant negative relationship with average daily position, for all fish (i.e. all batches combined). Based on the visual representations (Figures 5.5 and 5.6), this was expected, as during the period between 14 November and 12 December 2014 when river inflow increased markedly on three different occasions, many of the individuals made large movements. River inflow also had a significant positive relationship with the length of estuary used by the individuals within the lower and upper batches. Effectively, an increase in river inflow (with a 2-day lag) caused individuals to move greater distances downstream towards the mouth.

River inflow may affect the distribution and movement of estuarine fish by directly acting on an individual causing it to move, or causing marked changes in water chemistry (particularly salinity and temperature) that cause individuals to move to areas where conditions are more favourable (Heupel and Simpfendorfer 2008). Although the increased flow rates may have directly influenced the distribution of juvenile *R. holubi*, the significant relationships are more likely linked to a decrease in salinity. Although *R. holubi* is a good osmoregulator (Blaber 1973), the sudden increase in river inflow may have caused a distinct decrease in salinity making it necessary for certain individuals to move down the estuary (towards the mouth) to areas offering more favourable conditions.

River inflow has been shown to influence the distribution and movement of several estuarine fish species. Estuary perch *Macquaria colonorum* made large-scale downstream movements in response to increased freshwater inflow in the Shoalhaven Estuary (south-eastern Australia) (Walsh *et al.* 2013). Movement of *Acanthopagrus australis* in the Georges River Estuary (Australia) was related to intermittent freshwater inflow, however movement decreased during these events (Gannon *et al.* 2015). In contrast to the present study, *A. butcheri* individuals moved from the Gippsland Lakes (Australia) into the adjacent rivers for a brief period after river flows increased (Hindell *et al.* 2008).

This study has shown that river inflow plays an important role in the movement behaviour of juvenile *R. holubi*. Therefore, anthropogenic alterations in river inflow, such as excessive water abstraction for agricultural practices, and the building of dams, weirs and bridges, are likely to have direct and indirect effects on the distribution and movement of this species. These effects will be discussed in more detail in Chapter 6.

River inflow generally has a positive relationship with rainfall (i.e. river inflow increases with increased rainfall). Rainfall has been reported to affect the movement of fish in estuaries. For example, yellowfin bream *Acanthopagrus australis* switched their daily activity rhythm from diurnal to nocturnal during periods of rainfall in the Georges Estuary (Australia) (Payne *et al.* 2012). In this study, however, rainfall had no significant effect on daily position or the length of estuary used by juvenile *R. holubi*.

Salinity

Salinity has been recognised as a primary factor influencing the movement and distribution of fish within the estuarine environment (Whitfield 1999; Kerwath *et al.* 2005; Harrison and Whitfield 2006; Sakabe and Lyle 2010; Walsh *et al.* 2013). Moreover, the ability to adjust to changes in salinity is the most essential adaptation required by estuary-associated fishes (Panikkar 1960). In this study, salinity was not recorded, so the influence of salinity on the movement and distribution of juvenile *R. holubi* could not be assessed. Although other variables in this study (e.g. river inflow) could be used as proxies for changes in salinity, actual salinity values would have provided a more complete assessment of movement behaviour. Therefore, future studies should focus research attention on the effect of salinity on the movement and distribution of juvenile *R. holubi*.

Barometric pressure

Previous studies have shown that barometric pressure can have an effect on the movement behaviour of some fish species. For example, the number of spotted grunter *P. commersonnii* present within the Great Fish Estuary was significantly correlated with barometric pressure (Childs *et al.* 2008b). A sudden drop in barometric pressure was associated with all juvenile blacktip sharks *Carcharhinus limbatus* moving to deeper water just prior to the arrival of Tropical Storm Gabrielle in the Terra Ceia Bay (Gulf of Mexico) (Heupel *et al.* 2003). Furthermore, barometric pressure has been suggested by the recreational fishing community to be one of the main reasons for variations in catches (Stoner 2004).

In this study, barometric pressure had no significant effect on juvenile *R. holubi* distribution or movement. Moreover, it was the only variable not to be included in any of the models, further emphasising the lack of influence that barometric pressure had on the movement of juvenile *R. holubi*.

5.4.3 Conclusions

The geophysical cycles which influenced the movement patterns of juvenile *R. holubi* included diel and tidal cycles, as evidenced by the Fourier analysis of the presence/ absence (PA) data. Fourier analysis of the average receiver visited (ARV) data, however, did not show movement patterns related to geophysical cycles (with the exception of Fish 3 and 5). The high residency of juvenile *R. holubi* in relation to the resolution of the deployed receiver array meant that fine-scale movements were difficult to detect. Future studies should consider the use of manual tracking to determine fine-scale movements in relation to tidal and diel cycles. This would allow for a more complete picture of how these geophysical cycles influence the movement of this species. The analysis of the juvenile *R. holubi* movement patterns in relation to geophysical cycles did, however, further emphasise the high residency of juvenile *R. holubi*.

River inflow and water temperature were the environmental variables which influenced the movements and distribution of juvenile *R. holubi* to the greatest extent. Increased river inflow caused individuals to use larger portions of the estuary. Increased water temperature, particularly river temperature, caused individuals to shift their distributions downstream towards the mouth of the estuary. The inclusion of some variables in the models from different batches (particularly the lower and middle batches), however, may not have been appropriate due to the high delta AIC (Δ_i) values. This suggests that other variables may have been more appropriate (e.g. salinity or prevalence of predators). Future studies should, therefore, consider the influence of salinity and other abiotic and biotic variables on the movements of juvenile *R. holubi*.

The findings of this study have implications for the management of this species. The high residency of juvenile *R. holubi* means that this species may be susceptible to local depletion from fishing pressure. Conversely, the high residency levels also make the closure of areas to exploitation an attractive management strategy for this species. The high dependence of *R. holubi* on estuaries as nursery areas means that declines in abundance could occur due to habitat degradation. Management of estuaries and their catchment areas should, therefore, be a priority for the conservation of this species. Finally, because of the relationship between *R. holubi* distribution and water temperature, climate change may be an important factor with regard to changes in this species distribution, both within estuaries and along the coast. These issues will be discussed in more detail in Chapter 6 (General Discussion).

CHAPTER 6

GENERAL DISCUSSION

Knowledge on the movement behaviour of a species is fundamental to understanding its ecology and implementing effective management strategies (Meyer *et al.* 2000). Much research attention has focussed on the recruitment of *Rhabdosargus holubi* into estuaries after spawning, however, little information is known about their movement behaviour within estuarine systems, hence the necessity for this study. Based on the transmitter-effect experiment, which showed that the presence of transmitters had no significant adverse effects on individuals, acoustic telemetry was deemed well suited to achieving the overall aim of gaining a better understanding of the spatial ecology of juvenile *R. holubi* in the Kowie Estuary. The key findings of the telemetry study were:

(1) Estuaries are important nursery areas for the juvenile life history stage of *R. holubi*.
(2) Juvenile *R. holubi* showed high residency, particularly to the areas at which they were tagged and released, however the degree of residency differed among the batches.
(3) Diel and tidal cycles influenced the movement of several juvenile *R. holubi*, however, the lack of patterns for most of the individuals further emphasised the high residency of this species.
(4) River inflow and water temperature significantly affected the movement behaviour of juvenile *R. holubi*.

This chapter focuses on these key findings and the implications that they may have on the ecology and conservation of *R. holubi*.

6.1 Residency within estuaries

Juvenile *R. holubi* displayed high residency in and site fidelity to their tag and release sites within the Kowie Estuary. Compared with other South African estuary-associated species that have been studied using acoustic telemetry (e.g. dusky kob *Argyrosomus japonicus* (Cowley *et al.* 2008), white steenbras *Lithognathus lithognathus* (Bennett *et al.* 2015), and spotted grunter *Pomadasys commersonnii* (Childs *et al.* 2008b)), this species exhibited the highest residency levels. This may be explained by the trophic ecology, habitat preference, and the relatively small size of this species.

Movement behaviour of estuarine fish can be related to their respective trophic position. The movements of predatory species (particularly piscivores) often mirror those of their preferred prey species up and down the estuary, while species which feed on sedentary prey generally maintain their position within habitats of higher prey abundance. For example, *A. japonicus* (a piscivore) made relatively large movements up and down the Great Fish Estuary, which were related to the movement of dominant prey species (Næsje *et al.* 2012). In contrast, *L. lithognathus* (a macrobenthivore) displayed long-term residency and site fidelity in the lower reaches of the Kariega and Sundays estuaries, which was suggested to be driven by the high macroinvertebrate prey availability in this region (Bennett *et al.* 2015). Similarly, the high use areas occupied by *P. commersonnii* (also a macrobenthivore) in the lower reaches of the Great Fish Estuary coincided with the highest abundance of mud prawn *Upogebia africana*, a preferred prey item. Juvenile *R. holubi* are omnivorous with a preference for submerged macrophytes (Blaber 1973). Therefore, the high residency of juvenile *R. holubi* in the Kowie Estuary was likely related to the abundance of submerged macrophytes.

The use of structurally complex habitats decreases the risk of predation (Næsje *et al.* 2012). Consequently, the use of areas with submerged macrophytes by juvenile *R. holubi* has the added advantage of increased predator avoidance, as individuals hide in the plant structure making them more difficult to detect and capture (Whitfield 1984). Predator avoidance through the use of submerged macrophytes may, therefore, be another reason for the high residency of juvenile *R. holubi*.

Body size of an individual is also a factor which may affect area use. For example, there was a positive relationship between fish size and core area of *P. commersonnii* in the Great Fish Estuary (Childs *et al.* 2008b). Juvenile *R. holubi* in this study spent extended periods of time around the area adjacent to a single receiver (i.e. less than one kilometer of estuary length used), which is generally a smaller area than that used by other larger species tracked in South African estuaries (e.g. *A. japonicus*). Increased area use with increased body size may be due to (1) the increased resource requirements to sustain a larger individual and (2) the decreased relative cost of swimming a certain distance (i.e. smaller individuals would use more energy to get from one place to another) (Kramer and Chapman 1999). The resident behaviour exhibited by juvenile *R. holubi* in this study may be due, in part, to their relatively small size.

Although high levels of residency were recorded among all batches, this attribute did vary, with individuals from the upper batch generally using a significantly greater length of the estuary compared to the lower batch. Differences in space use among the batches may have been due to the higher abundance of submerged macrophytes (i.e. increased food availability and reduced predation risk) near the lower batch release site compared with the batches higher up the estuary. Another reason for the differences among the batches may be related to the width of the estuary, with the upper reaches generally being narrower than the lower reaches. The relatively narrow upper reaches would mean that individuals in this region would have to use a greater length of the estuary in order to cover the same area compared to individuals in the wider lower reaches.

The differences in space use among the batches illustrated the importance of tagging individuals along the length of the estuary. If the sample of fish was tagged in a single area in this study, the findings would not have been a comprehensive representation of their space use. Therefore, future telemetry studies should strive to tag and release individuals across the entire study area, where possible.

Several juvenile *R. holubi* showed diel and tidal movement patterns, however the fine-scale movements in relation to the spatial resolution of the receiver array meant that alternate techniques (e.g. manual tracking) may have been better suited to understanding the influence of geophysical cycles on their movement. The results of this section did, however, further emphasise the high residency of this species.

Resident behaviour has been suggested to have an ecological advantage as individuals become familiar with a specific area (Baras *et al.* 2002). Familiarity with an area allows for increased efficiency in resource utilisation and reduced predation. In the case of juvenile *R. holubi*, familiarity with an area would allow individuals to learn where suitable submerged macrophyte abundance can be located.

6.2 Dependency on estuaries

It is widely accepted that estuaries are important nursery habitats for juvenile fish. Beck *et al.* (2001) proposed that a habitat can be regarded as a nursery if it "contributes disproportionally to the production of individuals that recruit to adult populations" compared to other habitats with juveniles from the same species. The high dependence on the estuarine environment and the fact that once individuals left the estuary they generally did not return during this study, emphasises the nursery role of estuaries and the biphasic life history of *R. holubi* (Whitfield 1999; Beck *et al.* 2001).

Estuaries are not the only habitats which have been shown to provide nursery areas for juvenile *R. holubi*. Beckley (1985b) found that intertidal rock pools provided nursery areas for juvenile *R. holubi* (42 – 149 mm) along the Eastern Cape coastline, however it was suggested that these areas were only used by individuals that failed to recruit into estuaries. Whitfield and Pattrick (2015) observed the utilization of estuarine and/or marine littoral habitats by the juveniles of common coastal fish species in the Eastern Cape, but found that juvenile *R. holubi* did not exhibit any form of dependence (partial or complete) on marine littoral habitats. Therefore, estuaries seem to provide the only significant nursery area for juvenile *R. holubi*.

Juvenile *R. holubi* exhibited limited connectivity with the marine environment, with only one individual returning to the estuary after visiting the marine environment. Similarly, only one out of seven juvenile *L. lithognathus* individuals tracked in the Great Fish Estuary visited the marine environment (Bennett *et al.* 2011). The low connectivity with the marine environment further emphasises the dependence of juvenile *R. holubi* on the estuarine environment.

6.3 Threats to *Rhabdosargus holubi*

Factors that threaten fish vary and can differ from one region to another (Bruton 1995). Knowledge of fish movement behaviour can provide valuable insights regarding the potential impacts of these threats, as well as allowing the implementation of correct management decisions. Common threats to estuary-associated species include 1) habitat degradation, 2) climate change and 3) fishing pressure. Although many factors may threaten *R. holubi* directly or indirectly, this section will focus on these three important threats.

6.3.1 Habitat degradation

Estuaries are among the most degraded habitats worldwide because of the large number of people who have colonised coastal environments (particularly the mouth region of estuaries) throughout history (Edgar *et al.* 2000). Estuarine degradation, particularly within South Africa, is an ongoing process and appears to be accelerating (Whitfield 1986; Cyrus 1991; Whitfield and Cowley 2010). Since *R. holubi* is highly dependent on estuaries during their juvenile life-history stage (Blaber 1973), the loss of this environment would have lasting adverse effects on their abundance (Whitfield 1994a).

Of particular importance in South Africa is the condition of estuarine catchment areas, as the mismanagement of these areas is the most important factor accounting for the degradation of estuarine habitats (Whitfield and Cowley 2010). Two characteristics of mismanaged catchments include (1) excessive freshwater abstraction and (2) increased downstream transport of sediment loads. Firstly, excessive water abstraction leads to estuaries becoming marine-dominated in terms of salinity and other physio-chemical properties due to a lack of freshwater input (Cyrus 2000). This can have serious consequences for the health of the system, as was the case in Lake St Lucia where hypersaline conditions resulted in the direct loss of species (Whitfield 2005). As *R. holubi* is a good osmoregulator, hypersaline conditions may not have direct adverse effects on individuals; however, indirect effects may include reduced resource availability due to the reduction in abundance of preferred prey species unable to tolerate such conditions. Furthermore, reduced freshwater input could adversely affect the abundance of *R. holubi* in estuaries as this species relies on olfactory cues to recruit into estuaries (James *et al.* 2008b). Secondly, increased sediment load would adversely affect *R. holubi* through a probable reduction in submerged macrophytes. The deposition and the consequent burial of the plants under the sediment, as well as increased turbidity associated with high sediment loads could reduce plant growth rates and density due to a decline in the penetration of photosynthetic active radiation within the water column (Whitfield 1999).

With the ever-increasing human population and the popularity of estuaries for residential and industrial development, it is likely that urbanisation (particularly in the lower reaches) will continue to negatively impact estuaries and associated environments. The construction of residential and industrial structures on the banks of estuaries, together with the building of dams, weirs and bridges, can lead to the loss of important habitats within estuarine environments (Whitfield and Cowley 2010).

Water pollution in estuaries originates from a number of sources including runoff from urban and industrial areas, and insecticides and herbicides from agricultural land, both of which can be toxic (Cyrus 1991). The lower reaches of the Kowie Estuary are situated within the town of Port Alfred, while some of the land adjacent to the middle reaches and catchment areas used for agricultural purposes. The presence of the urban and, to a lesser extent, agricultural areas along the Kowie Estuary make it susceptible to water pollution, which may directly affect the abundance of *R. holubi* through mortalities or indirectly through reduced resource availability of preferred food sources.

For a number of estuary-associated species, particularly *R. holubi*, the widespread degradation of estuaries could have severe consequences as there is a close link between the availability of estuary nursery areas and the abundance of adults in the marine environment (Mann and Pradervand 2007). Well-conserved estuaries, however, are able to facilitate rapid recoveries in fish stocks in adjacent environments (Whitfield 1997), therefore the protection of suitable estuarine environments is essential for maintaining healthy populations of *R. holubi*.

6.3.2 Climate change

Climate change is expected to alter the physical structure and biological characteristics of estuaries, which is likely to have implications for estuary-dependent fish species (Whitfield and Cowley 2010). In particular, climate change is likely to have an impact on the abundance, distribution and movement behaviour of *R. holubi* within estuaries. In this study, river inflow and water temperature significantly affected the movement and distribution *R. holubi*, therefore this section will discuss the effects that climate change may have on these variables and the implications that these could have for *R. holubi*.

Water temperature

Most fish are poikilothermic with small changes in water temperature able to cause alterations in their behaviour and physiological processes (Beitinger and Fitzpatrick 1979;

Walsh *et al.* 2013). Climate change is expected to increase mean water temperatures over time. However, a shift in wind patterns is also expected to intensify the periods of upwelling along the Eastern and Western Cape coastlines causing sporadic, rapid cooling of water temperatures in the lower reaches of estuaries (Potts *et al.* 2015).

In this study, the general increase in river water temperature was associated with movement of individuals, particularly from the upper batch, towards the lower reaches of the estuary. Furthermore, decreased sea water temperature likely forced individuals in the lower reaches towards the middle reaches of the estuary. Such shifts in position may have been due to the temperature tolerances of juvenile *R. holubi*, particularly when salinity was low. With a general increase in water temperatures (particularly in the upper reaches) over time due to climate change, according to the findings of this study, *R. holubi* distribution within estuaries would be expected to shift towards the lower reaches of the estuary. However, intensified upwelling would rapidly decrease sea temperature causing individuals to move towards the middle reaches of the estuary. In effect, this may have a 'squeezing' effect on the distribution of *R. holubi* towards the middle of the estuary. This 'habitat squeeze' effect may impact juvenile *R. holubi* through increased intra-specific competition, increased susceptibility to exploitation in this region, and the loss of potential foraging habitat where conditions are not favourable.

As the distribution of *R. holubi* along the coastline of southern Africa is primarily determined by water temperature (Blaber 1973), increased water temperature associated with climate change should result in a shift in their distribution. Distributional shifts of certain tropical species have already been observed in estuaries within the Eastern Cape (James *et al.* 2008a). Increasing water temperatures could, therefore, cause a shift in the core distribution of the species from the Eastern Cape towards the Western Cape. The geography of South Africa and presence of the cool Benguela region in the west, with its paucity of estuaries, could restrict the westward shift of *R. holubi*, thus decreasing the total distributional range of this species. The decreased distribution would decrease the number of estuaries available for recruitment, thereby reducing the total abundance of *R. holubi*.

River inflow

River inflow has a strong influence on the functioning of estuaries (Whitfield 2005). As river inflow is closely linked to rainfall, the effect of climate change on the hydrological cycle is likely to play an important role in the functioning of estuaries. In South Africa the effect of climate change is expected to vary from one region to another. For example, in the Western Cape rainfall is predicted to decrease, whereas summer rainfall in the eastern parts of the country is expected to increase with extreme rainfall events becoming more frequent (Hewitson and Crane 2006; Engelbrecht *et al.* 2011).

Reduced river inflow over extended periods may cause estuaries to remain closed for longer and open for shorter periods, decrease the number of permanently open estuaries, and reduce the transportation of nutrients, sediment and detritus into the estuarine and coastal regions (James *et al.* 2013). This would adversely affect *R. holubi* by decreasing the availability of suitable nursery habitats, and reducing recruitment cues and opportunities into estuaries. Increased river inflow over extended periods may increase the sediment load transported into estuaries and the coastal zone (James *et al.* 2013). This could adversely affect *R. holubi* by the suspended particles clogging their gills, smothering the benthos (meaning reduced resource availability), and increasing turbidity which could affect resource detection and predator avoidance. Decreased river inflow may also lead to large portions of estuaries becoming hypersaline, which could have direct and indirect effects on juvenile *R. holubi* through the loss of available habitat and/or the reduction in available resources (i.e. submerged macrophytes).

6.3.3 Fishing pressure

Primarily caught by recreational and subsistence fishers, *R. holubi* is an important fishery species in many Eastern Cape estuaries (Gotz and Cowley 2013). Fishing effort in estuaries has become increasingly significant in recent years, particularly since the ban on beach driving in 2002, which has led to the depletion of a number of estuary-associated species (e.g. *Argyrosomus japonicus*) (Potts *et al.* 2005). Consequently, the decrease in other popular fishery species has led to increased catches of *R. holubi* over the same period. For example, Pradervand and Baird (2002) surveyed the line-fishery in the Sundays Estuary (South Africa) and found that *R. holubi* contributed 4.3% to the total catch, whereas Cowley *et al.* (2013) showed that *R. holubi* increased to 30.1% of the total catch in the same estuary.

Juvenile *R. holubi* in this study were highly resident within the area where they were tagged and released. This high level of residency, as well as their high catchability, makes this species susceptible to localised depletion. Area closures within estuaries which protect important habitats (e.g. submerged macrophytes) would, therefore, likely be the most effective management strategy for the conservation of this species, particularly as estuaries have had relatively little protection compared to the marine environment (Childs *et al.* 2008c). Although, the status of *R. holubi* is considered healthy (Cowley *et al.* 2013), the increased catches in the recent past may lead to a decline in the abundance of this species. Effective management of this species should therefore be prioritised in order to mitigate future declines of this species.

6.4 Future research and concluding remarks

The findings of this study have added considerable insights into the estuarine ecology of juvenile *R. holubi*, and provided further evidence that acoustic telemetry is an ideal technique to study the movement behaviour of fish. However, although this study provides a good knowledge base on the movement behaviour of juvenile *R. holubi* in an estuarine environment, future research could use different techniques and focus on a number of aspects of their ecology not covered in this study. Firstly, the relatively small size of the transmitters (7 mm Ø) used in this study meant that the telemetry experiment ran for approximately four months; therefore meaning that it was not possible to address seasonal movement patterns. The use of transmitters with longer battery lives would allow for the detection of seasonal movement patterns. Secondly, a future study could focus on the movement of *R. holubi* within a temporarily open/closed estuary and compare the results with the findings from this study (in a permanently open estuary). Thirdly, manual tracking could be used to determine fine-scale movements that the receiver array used in this study was unable to detect. This is particularly the case when focusing on the effect of geophysical cycles on the movement patterns of *R. holubi*. Finally, the effect of salinity on the movement and distribution of juvenile *R. holubi* could be a prime research focus as this variable, along with water temperature, is one of the most important environmental drivers affecting the movement and distribution of fish in estuaries (Whitfield 1999).

This study has emphasised the importance of estuarine environments to *R. holubi*. The loss or reduction of healthy estuarine habitats may lead to significant declines in the abundance of this species. Therefore, it is imperative that effective management strategies are put in place to conserve healthy estuarine systems, as well as their catchment areas.

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