USING ACTION CAMERAS TO ASSESS HABITAT USE BY *PSEUDOBARBUS AFER* AND *SANDELIA CAPENSIS* IN THE SWARTKOPS RIVER, EASTERN CAPE, SOUTH AFRICA

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Abstract

Currently, freshwater systems are facing various threats, freshwater biota are declining and there is an increased need to monitor freshwater fauna and flora using non-invasive methods. This thesis aimed to evaluate the potential of using action cameras as a tool for the monitoring of freshwater fish populations and the collection of habitat utilisation data. This evaluation was conducted in headwater tributaries of the Swartkops River in South Africa, using two threatened species that have not been extensively studied, *Pseudobarbus afer* (Peters, 1864) and *Sandelia capensis* (Cuvier, 1831). The aims of this study were to: (1) assess the use of underwater video analysis (UWVA) using action cameras (videos and still photographs) in comparison to estimates derived from snorkel surveys, to estimate the abundance of *P. afer* in headwater streams; (2) use estimates derived from UWVA (videos and photographs) to assess the habitat use of two imperilled species, *P. afer* and *S. capensis*; and (3) assess habitat use by these two species in the presence of an artificial habitat.

This work demonstrated that: (1) estimates derived from videos and photographs were strongly correlated to estimates derived from snorkel surveys, provided multiple cameras were used; (2) estimates derived from videos and photographs were not significantly different to estimates derived from snorkel surveys; (3) a filming period of 15 minutes was sufficient at detecting 0.9 of the cumulative mMaxN (mean MaxN) within one of the five habitats; and (4) still photographs, which are less time consuming to process than videos, could be used in preference to videos. Based on these findings, techniques using action cameras to assess habitat utilisation and behaviour *in situ* of the two-focal species, were developed using six habitat types (inflow, outflow, woody debris, fern root, middle and artificial) in four pools. It was demonstrated that: (1) there was a significant difference in proportional occupancies across habitats for both *P. afer* and *S. capensis*; (2) *Pseudobarbus afer* were mainly observed schooling in the middle of the pool and feeding on detritus material in fern root, woody debris and off the artificial structure; and (3) *Sandelia capensis* rapidly colonised the artificial structure and were observed utilising it for refuge.

In conclusion, this work demonstrated that still photographs from action cameras can be used in place of videos to estimate the abundance of freshwater fishes and assess their habitat use and behaviour in clear headwater streams. This work also demonstrated how action cameras could be used to evaluate the effect of the introduction of artificial habitat as a restoration measure for headwater fish communities.

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CHAPTER 1:

GENERAL INTRODUCTION, THESIS BREAKDOWN AND STUDY AREA

1.1 Introduction

Freshwater biodiversity is higher than would be expected considering the small area of the Earth covered by fresh waters, less than 1% (Dudgeon *et al.*, 2006; Balian *et al.*, 2008), however, freshwater fauna represents 9.5% of known fauna, making fresh waters a hotspot of biodiversity (Strayer & Dudgeon, 2010). Globally, many freshwater resources are facing anthropogenic threats such as the overexploitation of fresh water and fish species, water pollution, flow modification, destruction or degradation of habitats and invasion by non-native species (Dudgeon *et al.*, 2006). Furthermore, freshwater systems are more vulnerable to changes than their terrestrial equivalents and once impacted they may decline at a faster rate than terrestrial habitats (Stiassny, 1999) with damage, in most cases, being irreparable.

1.1.1 Threats to freshwater systems

Human populations are the greatest contributors to the threats faced by fresh waters and associated ecosystems (Meybeck, 2003). They are responsible for the overuse and misuse of water resources, and, without underlying problems being resolved, freshwater biodiversity will increasingly be at risk (Vörösmarty *et al.*, 2010). Globally, human dependency on freshwater resources is high and the survival of human settlements is dependent on having access to fresh water. Vörösmarty *et al.* (2010), in an assessment of human and biodiversity needs, showed that by the year 2000, 80% of the world's population lived in areas where freshwater biodiversity threats exceeded the 75th percentile. Furthermore, the authors showed that out of the world's 47 largest rivers, 30 were considered as facing moderate to high threat levels. The greatest threats to fresh waters are flow modification and the misuse of water resources, including the creation of croplands or diversion of water to crops, river fragmentation and fishing pressure (Vörösmarty *et al.*, 2010).

Freshwater fishes (freshwater and strictly peripheral species) are currently estimated at almost 13,000 species (2,513 genera), or approximately 15,000 when all species occurring in fresh or brackish waters are included (Lévêque *et al*., 2008). This estimate, represents almost half of the number of recognised fish species globally. The International Union of Conservation of Nature (IUCN) has estimated that approximately 25% of freshwater fish species globally, are considered threatened (Hinton-Taylor *et al.*, 2009). The publication of the IUCN first red data list, identified the most threatened freshwater fish species in South Africa (Skelton, 1987). Subsequent red list evaluations have identified that the increasing numbers of freshwater fishes in South Africa are being considered imperilled at each iteration (Tweddle *et al.*, 2009). Coke (1988) reviewed the history of freshwater fish conservation in South Africa, highlighting that early studies focussed on successfully introducing non-native fish species, mostly for recreational angling. Focus around the conservation of native fishes only became a priority in the late 1970s. Although, more recent works have examined impacts of non-native fish introductions on native fishes (Ellender & Weyl, 2014), knowledge of the biology and ecology of most of South Africa's native freshwater fishes remains sparse and concentrated on a few high-profile species. Due to new evidence based on the impact caused by non-native fishes, new legislation and policies have been issued regarding the use of freshwater ecosystems and their biota (van Rensburg *et al.*, 2011).

1.1.2 Cape Floristic Ecoregion

In South Africa, the Cape Floristic Region is recognised as a global biodiversity hotspot and is a conservation priority for freshwater biodiversity (de Moor & Day, 2013). The term Cape Fold Ecoregion (CFE) has been used in this thesis as it is more appropriate when discussing freshwater systems within the Cape Floristic Region (Thieme *et al.*, 2005). The CFE has a critical conservation status and, although the full extent of freshwater fish species is only now being fully documented (Ellender *et al.*, 2017), diversity and endemism of aquatic fauna within the region is particularly high (de Moor & Day, 2013). The CFE contains many range-restricted endemic freshwater fishes and, therefore, is considered as an area that has a high fish conservation priority (Ellender *et al.*, 2017). Currently, there is little knowledge on the biology and ecology of the CFE fish fauna. In addition, conservation of these fauna, and the long-term impact that human-mediated threats will have on freshwater fauna, is poorly understood. Without a good understanding of the biology and ecology of freshwater fishes, conservation initiatives could be ineffective. Therefore, there is a need for researchers to gain a better understanding of the biology and ecology of the ecoregion's freshwater fishes to design more effective and species-specific conservation plans.

The freshwater fish within the CFE face serious threats because of the presence of invasive non-native fishes, habitat destruction, pollution and genetic isolation (Skelton, 2001; Tweddle *et al.*, 2009). Tweddle *et al.* (2009) reported that 23 of the 24 endemic fish taxa found in the CFE were threatened by the presence of non-native fish, while 18 of these are also threatened by habitat loss. It was also found that there was a strong correlation between absence of native fishes and the presence of non-native fishes, namely bass, salmonids and other piscivorous fish species (Impson, 2007; Tweddle *et al.*, 2009, Marr *et al.*, 2012). Van der Walt *et al.* (2016) showed that out of a total of 41 rivers examined in the Olifants-Doring catchment in the CFE, 28 had invasive fish species n them. Furthermore, most of the remaining native fish populations were restricted to headwater streams above physical barriers preventing non-native fish invading further upstream. Nel *et al.* (2007) showed that the CFE contained the most threatened river ecosystems in South Africa, describing 21% of them as critically imperilled with only 20% of the rivers of the CFE still classified as intact. The high level of endemicity of fishes found within headwater streams of the CFE is due to the high levels of geographic isolation between fish populations (Linder *et al.*, 2010), resulting in unique fish assemblages (Skelton, 1987). For example, the Eastern Cape redfin minnow, *Pseudobarbus afer* (Peters, 1864), is one of 14 distinct taxa of *Pseudobarbus* in the CFE (Ellender *et al.*, 2017) and this genetic radiation is due to the different taxa being isolated from one another (Swartz *et al.*, 2007).

1.1.3 Need for understanding fishes of the CFE

Understanding how fish utilise habitats, may aid in determining the conservation steps needed to protect native fish populations. Fish use a wide array of habitats within waterbodies depending on their life cycle specific needs (Schlosser, 1991). Heterogeneous environments offer numerous resources to fishes, which often allows coexistence of species with overlapping trophic levels or niches (Wilbur, 1980). Fishes will favour certain habitats depending on their needs, for example, the need to forage will favour a use of a habitat that provides food whereas the need for refuge will favour a habitat that provides shelter (Odling-Smee *et al.*, 2011). Once it is understood which habitats fish utilise throughout their life cycle, and for what they utilise these habitats, habitats could be artificially constructed to test whether fish will still utilise them. Conservation benefits linked to such approaches could include the use of artificial habitats to restore degrading habitats, thereby potentially improving biodiversity.

Artificial habitats can be created out of various substances ranging from PVC piping to concrete blocks to old structures (Stone *et al.*, 1991). Due to the high level of threats faced by river systems in the CFE, there may be a need for the construction of artificial habitats to increase population numbers of some fish species. Artificial habitats are mainly used within marine environments (Bohnsack & Sutherland, 1985; Lek & Guégen, 1999; Seaman & Sprague, 2001; Sherman *et al.*, 2002), however, there are many cases where artificial habitats have been used in fresh waters (Seaman & Sprague, 1991; Santos *et al.*, 2008; Cooksley *et al.*, 2012). Artificial habitats in freshwater ecosystems should only be introduced once a target species has been identified and post-deployment monitoring is obligatory to evaluate the outcome of the habitat introduction (Stone *et al.*, 1991). The use of artificial structures, may be particularly relevant, when working in areas with imperilled species or areas that are high in endemism such as headwaters. Artificial structures may help boost population numbers of imperilled species, however, they may only be successful if the fish species in question are facing degradation of habitat essential to their life-cycle.

Headwater streams in the focal study area for this thesis, the Swartkops River, Eastern Cape, South Africa (see Section 1.2), are characterised by the native fish *Pseudobarbus afer, Sandelia capensis* (Cuvier, 1831), *Enteromius pallidus* (Smith, 1841), *Glossogobius callidus* (Smith, 1937), and *Anguilla mossambica* Peters, 1852. This thesis focuses on *P. afer* and *S. capensis*. Both focal species (*P. afer* and *S. capensis*) are small-bodied species (so artificial habitat need not be big) and pools in these headwater streams are also small (artificial habitat needs to be small not to interfere with the natural habitat). With the increase in threats to freshwater fishes, especially in headwater streams, there is an increased need to monitor populations in these systems using techniques with the lowest impact.

1.1.4 Conventional fish sampling techniques

The need to utilise sampling methods that are both non-invasive and non-destructive is particularly relevant when working on systems that contain imperilled fish species (Ellender *et al.*, 2012). In headwater streams, fish populations are generally assessed using electrofishing (Snyder, 2003; Hickey & Closs, 2006), seine netting (Anderson *et al.*, 1995; Jordan *et al.*, 2008), fyke netting (Gerhardt & Hubert, 1991; Coggins *et al.*, 2006), snorkel surveys (Dolloff & Kershner, 1976; O' Neal, 2007; Jordan *et al.*, 2008; Ebner *et al.*, 2009) or using underwater video assessments (Ellender *et al.*, 2012; Wilson *et al.*, 2014). The advantages and limitations of each of these methods are summarised in Table 1.1. Methods, such as electrofishing, fyke netting and seine netting, which involve physical handling of the fish could remove their protective mucilaginous layer (Brydges *et al.*, 2009) and increase the risk of infection and stress (Snyder, 2004).

These examples demonstrate the benefits of sampling fish populations with techniques that cause minimal disturbance or alteration to fish behaviour. The use of action cameras can also reduce the amount of time required to sample a waterbody as some sampling methods can be lengthy such as the use of fyke or gill nets which will have to be placed in a waterbody for an extended period (Hubert *et al.*, 2013). In addition, action cameras are a cost-effective survey method and are now being manufactured to have high-resolution imagery and are incredibly versatile, which allows them to be used for sampling in various environments (Struthers *et al.*, 2015). Advantages and limitations are presented in Table 1.1.

While there are many studies that make use of UWVA for population assessments (Ellender *et al.*, 2012, Weyl *et al.*, 2013) or habitat utilisation, there is a lack of such studies in South Africa. Therefore, it is crucial to provide examples of the use of UWVA from South Africa to assess habitats favoured by imperilled species such as *P. afer* and *S. capensis* to raise the profile of the use of these techniques in South Africa and to collect data to better inform conservation practices.

This thesis evaluates the use of action cameras to assess habitat utilisation by two imperilled freshwater fishes, in two headwater tributaries of the Swartkops River in the Eastern Cape, South Africa. This will be done by: (1) assessing the effectiveness of UWVA in detecting fish abundance in comparison to snorkel surveys; (2) assessing the effectiveness of still photographs in comparison to UWVA and using these methods to observe *P. afer* and *S. capensis* looking at their habitat utilisation *in situ*; and (3) comparing natural habitat utilisation with the use of artificial habitats *in situ* by these two species.

1.1.6 Study species

The Eastern Cape redfin *Pseudobarbus afer* a cyprinid endemic to the Eastern Cape, (Fig. 1.1.) has been evaluated as Imperilled according to the 2007 IUCN Red List (Swartz & Impson, 2007). *Pseudobarbus afer* prefers clear rocky pools typically found in headwater streams. Fry and juvenile fish move in large schools while adults move in smaller groups. It breeds in summer in small riffles occurring in pools and attains a length not longer than 110 mm (Cambray, 1992).

Table 1.1. Advantages and limitations of several active and passive fish sampling techniques classically used to survey fish populations within fresh waterbodies.

Sample method	Advantages	Limitations	References
Electrofishing	▪ Immediate results ▪ Not selective ▪ May be compromised by environmental conditions (conductivity of water may be too high or too low)	▪ Various injuries can occur: spinal injuries, haemorrhaging in soft tissue, fractures in hard tissue (bone), respiratory failure ▪ Stress/trauma inducing ▪ Stunting of growth ▪ Mortality ▪ Inferences about behaviour cannot be made	Sharber and Carothers (1988); Dalbey *et al*. (1996); Thompson *et al*. (1997); Carline R (2001); Dwyer et. al (2001); Snyder (2003)
Passive gears (fyke or gill nets)	▪ Selective depending on mesh size (able to target specific species in question) ▪ Require little skill to operate ▪ Can be used in a variety of waterbodies ▪ Require physical contact with fish to release form net	▪ Selective depending on mesh size (not entirely feasible if sampling a wide size array of fish in one area) ▪ Mortality risk with entanglement gears (gill nets) ▪ May not be effective at targeting fish that are not active during their daily routine ▪ Inferences about behaviour cannot be made	Laarman and Ryckman (1982); Booth and Potts (2006); Hubert *et al*. (2012)
Push seine nets	▪ Immediate results ▪ These nets may provide for live release of fish ▪ Standardization of method removes sampler bias	▪ Size selective (mesh size influences species caught) ▪ Size of waterbody may affect efficiency of sampling technique. ▪ Requires a large amount of effort	Threinen (1956); Jessop (1985); Pierce *et al* (1990); Jordan *et al* (2008)
Snorkel survey	▪ Feasible sampling technique (only requires a mask and snorkel) ▪ Immediate results ▪ Non-invasive and non-harmful ▪ Effective at sampling rare or protected species as there is no handling. ▪ Inferences on habitat association can be made	▪ Sampler needs to know how to swim particularly in big/deep pools ▪ Requires good water visibility ▪ Fish behaviours may be altered in the presence of a snorkeler ▪ Bias in counts between different snorkelers	Dolloff *et al*. (1996); Mueller *et al*. (2003); O'Neal (2007); Jordan *et al*. (2008)
Action cameras	▪ Non-invasive and non-harmful ▪ Easily replicated ▪ Takes up less memory and battery life than the use of videos	▪ Limited field of view ▪ Time for analysis is lengthy (but not as long as time taken for photographs) ▪ Action cameras may be expensive and therefore there may be a financial limitation when using this method. ▪ Fish size not determinable (as opposed to the use of snorkelers estimating fish size)	Cappo *et al*. (2003); Ebner *et al*. (2009); Ellender *et al*. (2012); Wilson *et al*. (2014); Struthers *et al*. (2015)

Recent taxonomic work has described *P. afer* as a species complex and not just a single species (Chakona & Skelton, 2017). It was confirmed that one of the lineages of *P. afer* was more closely related to *Pseudobarbus phlegethon* (Barnard, 1938) and the three remaining lineages have been described as *P. afer* (Peters, 1864) restricted to the Baakens, Swartkops and Sundays river systems, Eastern Cape. *Pseudobarbus senticeps* (Smith, 1936) is restricted to the Krom River system, Eastern Cape, and *Pseudobarbus swartzi* (Chakona & Skelton, 2017) occurs in the Gamtoos and adjacent river systems (Chakona & Skelton, 2017).

Tweddle *et al.* (2009) showed that the main threats to *P. afer* were the introduction of non-native fishes and habitat destruction. The main stem of the Swartkops River has been invaded by at least five fish species (Ellender *et al.*, 2011) and in reaches of the river invaded by predatory non-native fish, population numbers of *P. afer* were greatly reduced (Skelton, 1993).

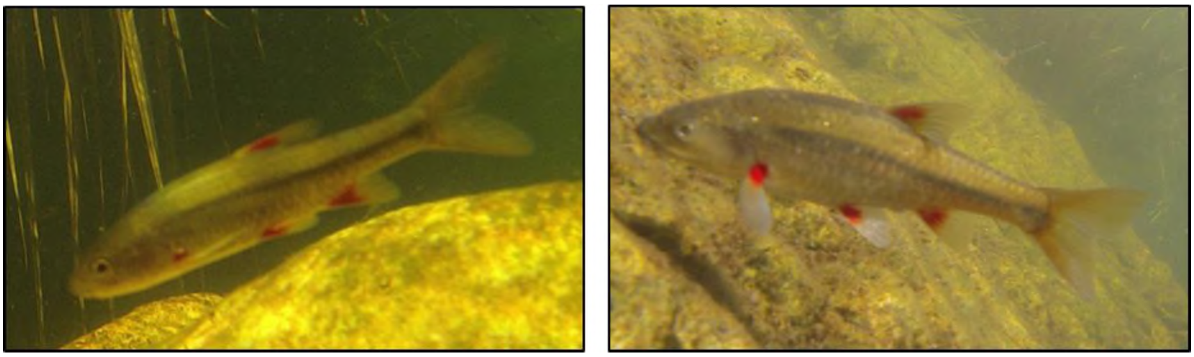

Figure 1.1. One of the two focal species for this thesis the Eastern Cape redfin minnow *Pseudobarbus afer* (photographs taken by B. Hannweg, 2016, Blindekloof).

The Cape kurper *Sandelia capensis* (Fig. 1.2.) is native to the Eastern and Western Cape and was evaluated as data deficient by the 2007 IUCN Red List due to incomplete taxonomic revision (Swartz & Impson, 2007). *Sandelia capensis* is found in the family **Anabantidae**, which has 33 species (Fishbase.org). Five lineages have recently been delineated for this species (Ellender *et al.*, 2017), however, the required taxonomic revision (Swartz & Impson, 2007) is still in progress. The genus *Sandelia* has two species; *S. capensis* and *S. bainsii* Castelnau, 1861.

Figure 1.2. One of the two focal species for this thesis the Cape kurper *Sandelia capensis* (photographs taken by B. Hannweg, 2016, Blindekloof).

Chakona & Swartz (2012) showed that *S. capensis* was found throughout the CFE but only in slower flowing waters of the lower elevations (< 400 m above sea level). The species is easily distinguishable by the black wavy bands found on its head. They attain a total length not more than 200 mm and breeding males change to a dark colouration (Skelton, 2001). Main threats to *S. capensis* include the introduction of non-native invasive species and habitat degradation through habitat modifications (Swartz & Impson, 2007).

1.2 Study area

The study area for this thesis are the Fernkloof (33°43’03.72’’S, 25°17’22.08’’E) and the Blindekloof (33°69’95.35’’S, 25°30’43.07’’E) tributaries (Fig. 1.3. & Fig. 1.4.) of the Swartkops River. Both streams are located within the Groendal Nature Reserve and are thought to be unaffected by human impacts and considered as episodic flowing only after rain has occurred.

Many non-native fishes have been introduced into the Swartkops River system including: largemouth bass *Micropterus salmoides* (Lacépède, 1802), smallmouth bass *Micropterus dolomieu* Lacépède, 1802, spotted bass *Micropterus punctulatus* (Rafinesque, 1819), bluegill sunfish *Lepomis macrochirus* Rafinesque, 1810, sharptooth catfish *Clarias gariepinus* (Burchell, 1822) and banded tilapia *Tilipia sparrmanii* Smith, 1840.

Ellender *et al.* (2011) showed that, of the non-native fish species in these river systems, *M. salmoides, M. dolomieu* and *C. gariepinus* were neither abundant nor widespread. However, the introduction of non-native invasive fishes has had a significant effect on endemic fishes such as *P. afer*. For example, Ellender *et al.* (2011) showed that *P. afer* was only found in a 1.7 km stretch of the Blindekloof River above a waterfall where the non-native fish had not invaded. It was also shown that *P. afer* were absent in suitable habitat below the waterfall. Due to *P. afer* having such a limited distribution, they are of conservation importance and, therefore, studying them is of importance.

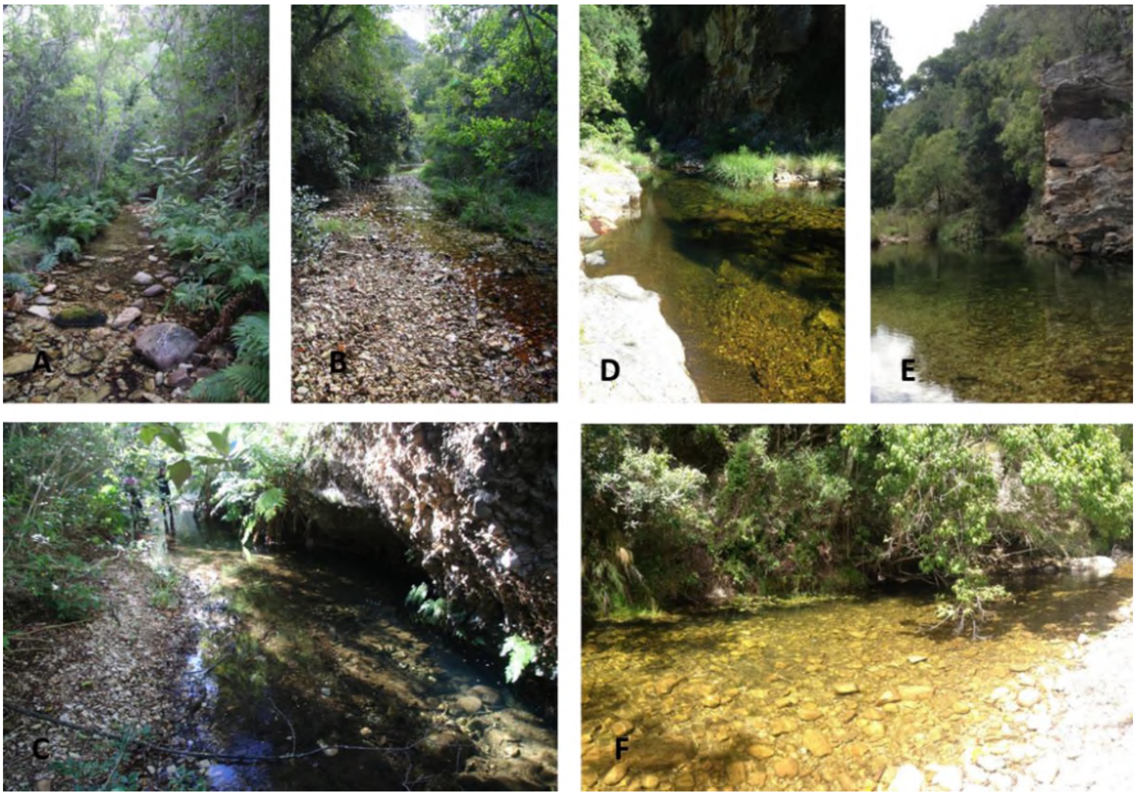

Figure 1.3. Photographs of what typical streams and pools look like in the Fernkloof tributary (A-C) and in the Blindekloof tributary (D-F). Photographs taken by B. Hannweg, Fernkloof and Blindekloof, 2016-2017.

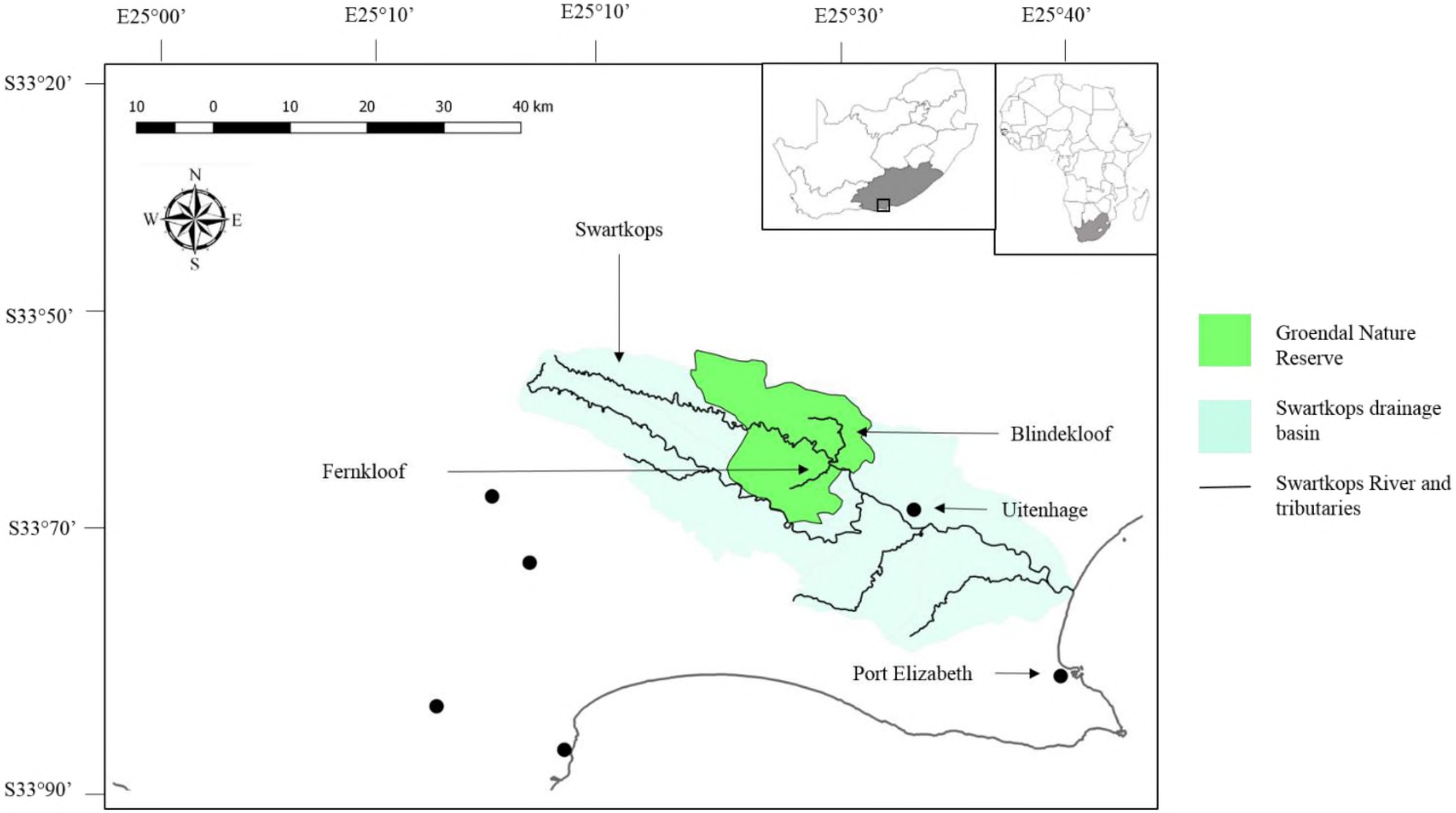

Figure 1.4. Location of the Swartkops drainage basin (blue) and the Groendal Nature Reserve (green) situated in the Eastern Cape, South Africa. Inserts show location in relation to Africa and South Africa. Black dots represent nearby towns with Uitenhage being the closest town to the study area.

1.3 Thesis outline

This thesis is divided into five Chapters. Chapter 1 introduces the overall topics of the thesis, describing the Cape Fold Ecoregion as a global biodiversity hotspot and the uniqueness of the freshwater fish fauna. Methods used to assess fish abundances are introduced with discussion on the need for non-harmful and non-destructive alternatives to these other methods. The focal fish species and study areas are then described. Chapter 2 compares three non-destructive, non-harmful methods (snorkel surveys, videos and still photographs) for assessing fish abundance. This is done *in situ* with the use of action cameras. Chapter 3 addresses habitat utilisation and preference of *P. afer* and *S. capensis* by defining fish behaviour and, thus, habitat selection. Chapter 4 investigates the response of *P. afer* and *S. capensis* following the introduction of an artificial habitat into pools within a headwater tributary of the Swartkops River and quantifies their utilisation of the artificial habitat. Lastly, Chapter 5 draws conclusions from the thesis addressing the findings from each data chapter as well as future uses and recommendations for each of the methods used.

CHAPTER 2

A COMPARISON BETWEEN SNORKEL SURVEYS AND UNDERWATER VIDEO DERIVED ESTIMATES OF ABUNDANCE OF *PSEUDOBARBUS AFER* IN THE SWARTKOPS RIVER, EASTERN CAPE, SOUTH AFRICA

2.1 Introduction

South Africa's Cape Fold Ecoregion (CFE) contains the highest number of threatened freshwater fishes in southern Africa (Tweddle *et al.*, 2009) and is one of the most invaded areas globally with more than 25% of the fish diversity made up of introduced species (Leprieur *et al.*, 2008). This ecoregion has been classified to have a critical conservation status and, although the full extent of native freshwater fish diversity has not been fully documented, the diversity and endemism of aquatic fauna is particularly high (de Moor & Day, 2013; Ellender *et al.*, 2017). Due to the high level of threats facing freshwater systems and the high level of endemicity found within the aquatic fauna of the CFE, there are many fishes in this ecoregion that are under severe threat for extinction (Tweddle *et al.*, 2009). These threats are further compounded by species traits such as restricted geographical distributions and isolation within headwater streams (Ellender *et al.*, 2017). There is, therefore, a great need to monitor the abundance of freshwater fishes within these isolated areas to aid in better understanding their requirements and to implement more informed conservation measures. There is also a need to develop methods that assess abundance of these fish populations that are neither destructive nor harmful to fish populations (Gray *et al.*, 2002; Hickey & Closs, 2006; Jordan *et al.*, 2008) in fresh waterbodies and the advantages and drawbacks of these methods are highlighted in Table 1.1. Two such methods are the use of still photographs and videos.

Ellender *et al.* (2012) compared underwater video analysis (UWVA) using action cameras to electrofishing to determine the least destructive method for assessing fish abundance in headwater streams. They made use of a single, colour video camera mounted on a small metal stand connected via a cable to a shore-based Sony digital video recorder to conduct UWVA. Furthermore, an electrofisher was used in the same pools to assess which method yielded the higher estimate of two headwater stream fish species. The authors found that estimates derived from UWVA were higher than those for electrofishing and that UWVA had beneficial conservation implications as it was a non-destructive method and could be used for imperilled species.

Weyl *et al.* (2013) examined numerous sites within the Rondegat River in South Africa, prior to treatment with the piscicide rotenone, using electrofishing, snorkel surveys and UWVA. Each method yielded different count estimates based on the reach being sampled and the species captured. Overall snorkel surveys and UWVA detected more fishes than electrofishing. Electrofishing was, however, more effective than snorkel surveys or UWVA in detecting species that were cryptic, or structure orientated.

A major challenge faced in the use of action cameras is controlling for repeated sightings of individual fish that could potentially skew estimates of fish abundance. The MaxN metric *sensu* Cappo *et al.* (2003), the total number of individuals of a species seen in a single frame at a specific time, has been used widely in video analysis to account for this problem (Cappo *et al.*, 2003 Watson *et al.*, 2010; Ellender *et al.*, 2012; Wilson *et al.*, 2014). With the functionality that action cameras can film videos and take photographs simultaneously, a direct comparison between abundance estimates from videos and photographs are possible. It could be expected that the results from photographs would be similar to those from videos, and that the use of photographs may drastically reduce analysis time. It is important to note that regardless of the method used, visual monitoring of fish populations is dependent on the accuracy of underwater surveys, which are dependent on the environmental conditions of the waterbody; depth, temperature and water clarity (Dolloff & Kershner, 1976).

The analysis of videos and still photographs makes use of the MaxN metric, where relative abundance is defined as the total number of species seen in a single frame at a specific time (Ellender *et al.,* 2012). An explanation of MaxN metrics used in this thesis are provided in Table 2.1.

This chapter assesses the efficacy of using videos and still photographs derived from action cameras for evaluating populations in two headwater tributaries of the Swartkops River in the Eastern Cape, South Africa, by comparing population estimates of *Pseudobarbus afer* in pools, derived from these two methods, to estimates derived from snorkel surveys. This was done to verify the assumption that estimates derived from action cameras will be strongly correlated to estimates derived from snorkel surveys as well as to explore less destructive and less harmful methods for assessing populations of imperilled fish species. The objectives were to: (1) validate Ellender *et al.* (2012)'s recommendations that 15 minutes filming time was sufficient for optimal camera deployment time; (2) assess the correlation between MaxN estimates derived from UWVA from the middle camera, in each pool and snorkel survey estimates; (3) assess the correlation between MaxN estimates derived from UWVA, still photographs from the middle camera and snorkel survey estimates when normalised by pool area; (4) assess the correlation between MaxN estimates derived from UWVA and still photographs from all cameras in each pool and snorkel survey estimates normalised by pool area; and (5) assess the potential that multiple simultaneous camera deployments in a pool could yield MaxN densities equivalent to snorkel surveys of the pool. It was predicted that higher MaxN estimates would be derived with the use of action cameras than those derived by snorkel surveys and that MaxN counts from multiple cameras normalised by pool area would be a better estimate of fish densities than when only the one camera was utilised.

Table 2.1. An explanation of MaxN derivatives used in this Chapter and in consequent chapters.

Derivative	Explanation	Acronym
MaxN	Total number of species seen in a single frame at any given time	MaxN
Mean MaxN	Mean number of species seen over a certain time derived from the MaxN	mMaxN
Sum MaxN	Sum of species seen over a certain time derived from the MaxN	ΣMaxN

2.2 Methods and materials

2.2.1 Study area

Data collection took place *in situ* over a two-week period, 2nd – 14th of February 2016 in two headwater tributaries of the Swartkops River, the Fernkloof (33°43' S, 25°17' E) and the

Blindekloof (33°69’ S, 25°30’ E) Tributaries near the town of Uitenhage in the Eastern Cape, South Africa (Fig. 2.1.). Both streams fall within the Groendal Wilderness Area and are thought to be largely unaffected by human impacts. Pools along the Blindekloof and Fernkloof permanently hold water but can become isolated at very low flow.

Ten pools (three in the Blindekloof and seven in the Fernkloof) were selected based on the criteria that: (1) they contained *P. afer*; (2) were deep enough to snorkel; and, (3) had visibility suitable for UWVA (based on the turbidity readings of the water and if the water was clear enough for snorkeler to count fish at least a body length away). Pools were characterised as areas that were deep along the river with substratum that was visibly different to that in the inflow or outflow section i.e. it was not shallow and did not contain riffles. The inflow and outflow to each pool mainly contained boulders (> 256 mm in diameter) while substratum in each pool ranged from gravel (10 – 64 mm diameter) to cobbles (64 – 256 mm diameter) with pockets of detritus and decaying plant material.

Water physico-chemical parameters were measured using a HANNA HI98129 combo probe for pH, temperature and conductivity (HANNA Instruments, Inc., United States of America). A portable HANNA HI 98703 turbidity meter (HANNA Instruments, Inc., United States of America) was used to measure turbidity. Three measurements were taken for each parameter in each pool and these readings were averaged over each pool. The length of each pool surveyed was measured (m) along the longest axis. A mean width was determined from five width transects and surface area was then calculated as the product of length and mean width.

All data analyses were conducted using R 3.4.1 statistical software (R Development Core Team. 2016 through RStudio 0.99.902 (RStudio, Inc.) and MS Excel 2013 (Microsoft). Statistical significance was determined using $p = 0.05$.

2.2.2 Absolute estimates

Baseline estimates for absolute abundances of *P. afer* densities in each pool were determined by snorkel surveys. Snorkel surveys were conducted using the two pass, zig-zag method modified from Woodford *et al.* (2005). This method requires that the snorkeler swims in a right to left or left to right pattern. Snorkel surveys started at the tail end of the pool with the snorkeler swimming upstream in a zig-zag pattern. The snorkeler was required to swim in an upstream direction first to prevent the disturbance of substrate clouding the snorkelers vision as it gets washed down with the flow of the river. The number of fish observed were recorded on a slate. The observer then conducted a second pass swimming downstream using the zig-zag pattern again recording the number of fish observed by species. To standardise density estimates between pools, the number of fish counted in each pool were divided by the surface area of the pool and expressed as *P. afer*/m².

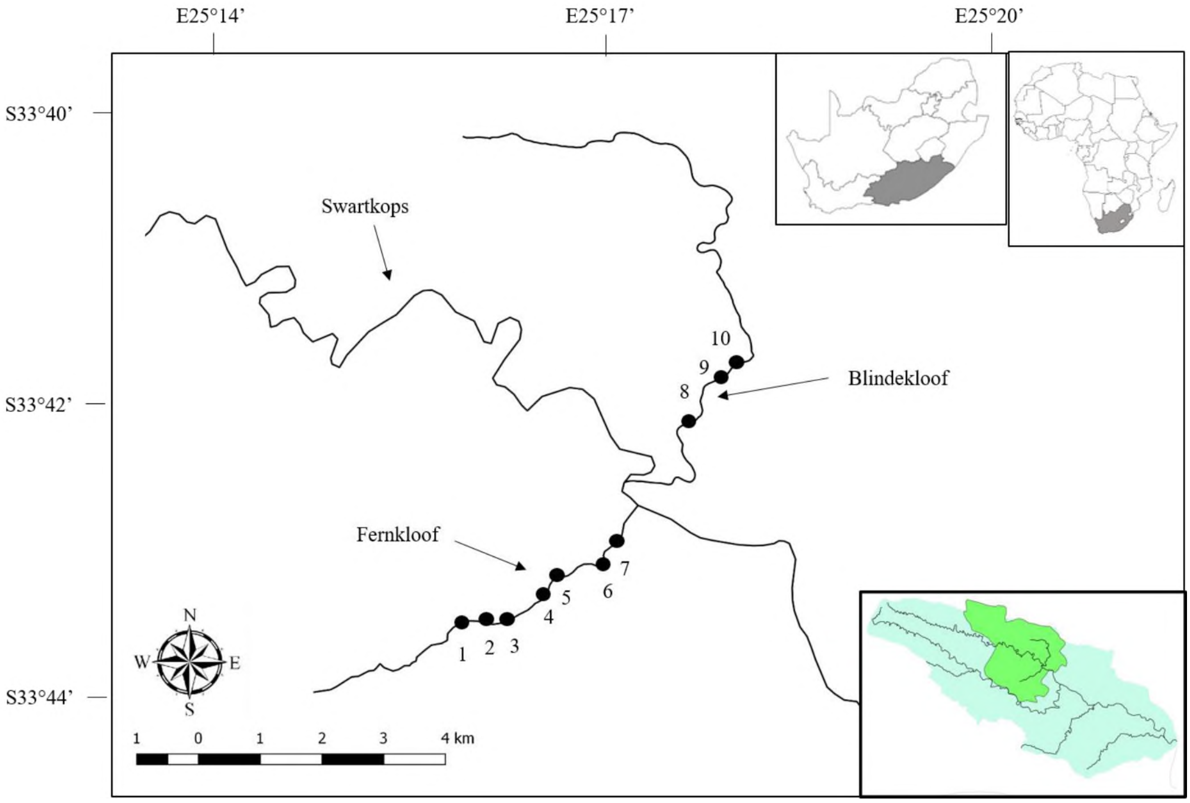

Figure 2.1. Study area in the Groendal Nature Reserve in relation to Africa and South Africa showing the location of Fernkloof and Blindekloof tributaries. The ten sampling sites (three in the Blindekloof and seven in the Fernkloof) are shown as black dots (•) and numbered 1-10. Arrows indicate where the Blindekloof (arrow on the right) and the Fernkloof (arrow on the left) tributaries are located. Map insert shows the location of the study site in relation to Africa and South Africa as well as the location of the study site in relation to the Swartkops drainage basin (blue) and the Groendal Nature Reserve (green).

2.2.3 Action cameras

Five GoPro Hero 3+ (GoPro Inc., United States of America) action cameras in waterproof housings were mounted on gorilla tripods and set at five preselected locations in each pool (SAIAB ethics clearance 25.4.1.7.5_2017-04) after the snorkel survey had been completed (Fig. 2.2.). The flow of the waterbody was low enough to not require additional weight on the camera tripods.

The five pre-selected locations included the inflow (head of the pool), woody debris (old branches and sticks which had fallen into the pool), the middle, fern root (an area where ferns were attached to the cliff on the side of the pool and fern roots hung in the water) and the outflow (tail end of the pool) and are numbered 1-5, respectively, in Fig. 2.2.

Camera placement in the pools ensured non-overlapping fields of vision. Following recommendations by Ellender *et al.* (2012), cameras were set to film for 18 minutes (including a three-minute acclimation period) at a frame rate of 720 fps and 12-megapixel resolution, concurrently taking photographs every five seconds.

2.2.4 Validation of filming period

To determine whether the 15-minute film time (excluding the acclimation period) recommended by Ellender *et al.* (2012) was appropriate, accumulation curves were fitted for each camera placement over the ten pools. Here, the MaxN at each time interval was calculated as a proportion of the total MaxN. The optimal deployment time was taken as the time at which 0.9 (or 90%) saturation had occurred for each camera placement. The 0.9 saturation time for each camera was tested for normality, followed by a t-test to test whether there were significant differences in time taken to reach 0.9 saturation between camera placements.

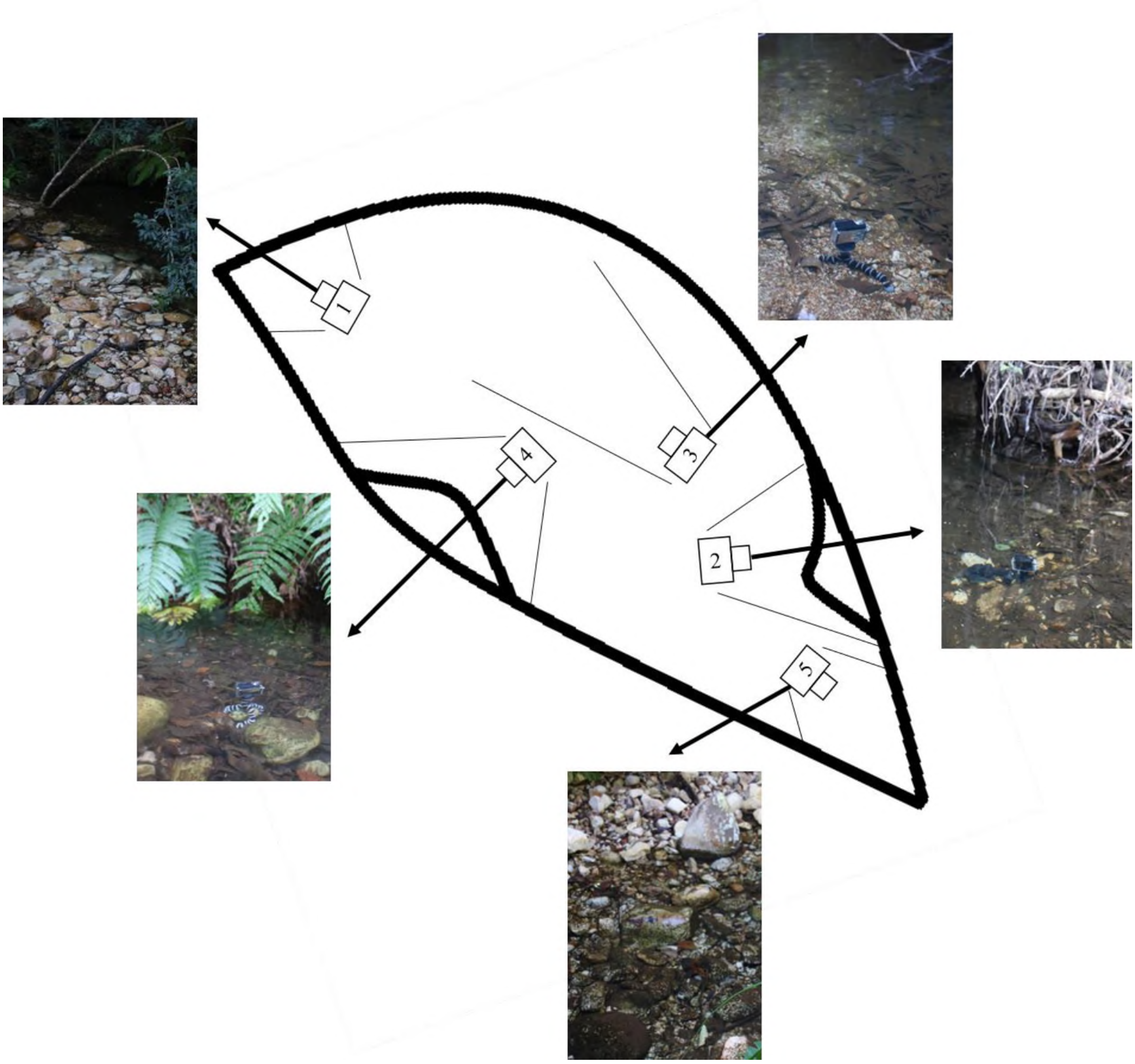

Figure 2.2. Schematic diagram of a typical pool within the tributaries of the Swartkops River and camera placement within each pool. Pre-determined locations were as follow: 1) inflow; 2) woody debris; 3) middle; 4) fern root, and 5) outflow. Lines indicate the area the approximate area that was covered by each camera. Photo inserts show what each area typically looked like.

2.2.5 Relative estimates

A MaxN value was derived for both videos and photographs. All videos were viewed in Windows Media Player (Microsoft Inc.). According to recommendations of Ellender *et al.* (2012), the initial 3-minute segment of each video was discarded as an acclimation period to allow the fish to become familiar with the disturbance of the snorkelling and the setting of the cameras. The three-minute acclimation period used here appeared to be sufficient as substrate had settled within the first three minutes allowing for clear visibility for both video and photo. For the remaining 15 minutes, MaxN was estimated as follows: (1) each 15-minute video was broken down into 30 intervals 30 seconds in length each; (2) the largest number of fish seen in a single frame within a specific 30 second interval was recorded as the MaxN for that time interval; and, (3) the highest MaxN recorded for the 30-time intervals were recorded as the overall MaxN for the deployment. Similarly, photographs were viewed in Windows Photo Viewer (Microsoft Inc.). The first 36 photographs, corresponding to the first three minutes of video, were discarded (acclimation period). Thereafter, a total of 180 photographs were viewed in batches of 6 photographs; equivalent to one video interval. The MaxN was then determined from the photo that contained the most fish for each batch of 6-photographs and recorded as the MaxN for each photo interval.

MaxN estimates derived from the videos were first computed only for the middle camera in each pool; following Ellender *et al.* (2012). In the study conducted by Ellender *et al.* (2012), only the middle camera was used for analyses as there was only one camera available for the study and it was placed in the middle of each pool. Video counts derived from the middle camera in each pool were compared to counts derived from snorkel surveys. Camera-based estimates derived from both video and still photographs from the middle camera were normalised by pool area and compared with snorkel-survey estimates normalised by pool area. Linear regression was used to describe the relationship between snorkel survey, video and still photo estimates and an ANCOVA was used to determine whether the slope of the linear regression was significantly different to 1. Following on from these analyses, MaxN estimates derived from videos and still photographs were computed for all cameras within the pool and compared to estimates derived from snorkel surveys.

MaxN estimates derived from videos from all cameras in a pool were then used to assess whether camera placement influenced the number of *P. afer* detected in each habitat. This was done as a baseline study for methods used in Chapters 3 and 4. An ANOVA was used to assess whether camera placement would influence the number of fish recorded. The results from these analyses were then used to assess which site within each pool was most appropriate in detecting the highest count of *P. afer*. Furthermore, MaxN estimates derived from all five cameras in the ten pools for both still photographs and videos were used in Chapter 3 to assess whether still photographs would yield counts comparable to those yielded from videos to use still photographs and reduce analysis time.

2.3 Results

2.3.1 Study area

A summary of the pool location, dimensions and water quality parameters are provided in Table 2.2. Pool lengths ranged from 5.4 m – 17 m. Pools in the Blindekloof tributary had surface areas less than 32 m^2 while pools in the Fernkloof tributary had surface areas less than 60 m^2. During the survey the water was very clear (turbidity mean ± standard deviation: 0.37 ± 0.25 NTU), with a mean temperature of 19.1 ± 0.9°C, a slightly acidic pH (range: 4.42-6.35) and had a low conductivity (274.23 ± 56.38 µs/cm). Flow between pools was low at the time of the survey.

2.3.2 Absolute abundances

Snorkel surveys yielded counts ranging from 10 to 60 *P. afer* per pool. Pool 5 had the highest snorkel count (60) and Pool 8 had the lowest, 10; both pools were found within the Fernkloof tributary. Four out of the ten pools had snorkel estimates that were in the thirties, with only two pools having higher numbers than 30 (excluding the maximum number, 60).

2.3.3 Validation of filming period

The accumulation curves derived from the proportion mMaxN estimates at each time interval are shown in Figure 2.3. (A - F). Figure 2.3. represents the different camera placements: Inflow (A), woody (B), middle (C), fern root (D) and outflow (E). The last graph (F) represents when all proportions were used over the five cameras. The earliest time interval at which 0.9 proportional occupancy of the cumulative mMaxN was reached was at four minutes in the wood debris habitat (Fig 2.3. D). The longest time to reach 0.9 of the cumulative mMaxN was 12.5 minutes in the middle habitat (Fig. 2.3. C). When all the cumulative mMaxN were summed over the five camera placements, a proportion of 0.9 of the cumulative mMaxN was reached within six minutes. There was no significant difference in time taken between the five different camera placements for the cumulative mMaxN to reach a proportion of 0.9 (*t-test;* d.f. = 4, t = 0.191, p = 0.86).

Table 2.2. Summary of habitat measurements, location and physico-chemical parameters (± standard deviation) for ten pools found within two headwater tributaries of the Swartkops River, Eastern Cape, South Africa.

Pool	**Location**	**Length (m)**	**Mean width (m)**	**Area (m²)**	**Water temperature (°C)**	**Conductivity (µs/cm)**	**Turbidity (NTU)**	**pH**
1	33°43'24.64"S, 25°17'7.80"E	13.0	2.6 ± 0.4	33.2	18.3 ± 0.1	344 ± 3	0.20 ± 0.03	5.56 ± 0.08
2	33°43'26.65"S, 25°17'5.28"E	17.0	3.0 ± 0.4	51.4	19.8 ± 0.1	336 ± 3	0.23 ± 0.02	6.23 ± 0.08
3	33°43'27.26"S, 25°17'3.72"E	10.0	3.5 ± 0.9	35.2	18.2 ± 0.1	205 ± 3	0.53 ± 0.09	5.12 ± 0.09
4	33°43'27.45"S, 25°17'3.33"E	10.0	3.1 ± 0.7	30.5	18.0 ± 0.1	233 ± 1	0.67 ± 0.43	5.15 ± 0.02
5	33°43'25.73"S, 25°17'7.12"E	6.6	4.7 ± 1.0	30.8	18.1 ± 0.1	346 ± 3	0.17 ± 0.02	5.54 ± 0.06
6	33°43'21.29"S, 25°17'13.38"E	9.4	3.3 ± 1.2	30.7	18.4 ± 0.1	338 ± 4	0.47 ± 0.22	6.09 ± 0.23
7	33°43'18.88"S, 25°17'15.17"E	5.4	3.1 ± 0.5	16.5	20.8 ± 0.1	241 ± 2	0.34 ± 0.04	5.55 ± 0.04
8	33°41'25.07"S, 25°18'29.42"E	8.9	4.1 ± 1.6	36.4	19.4 ± 0.1	236 ± 3	0.25 ± 0.02	4.93 ± 0.09
9	33°41'39.37"S, 25°18'35.96"E	6.8	2.5 ± 0.4	16.7	19.8 ± 0.1	233 ± 4	0.22 ± 0.08	5.18 ± 0.17
10	33°41'56.81"S, 25°18'14.37"E	7.4	2.1 ± 0.5	15.4	19.9 ± 0.1	230 ± 1	0.65 ± 0.36	4.84 ± 0.68

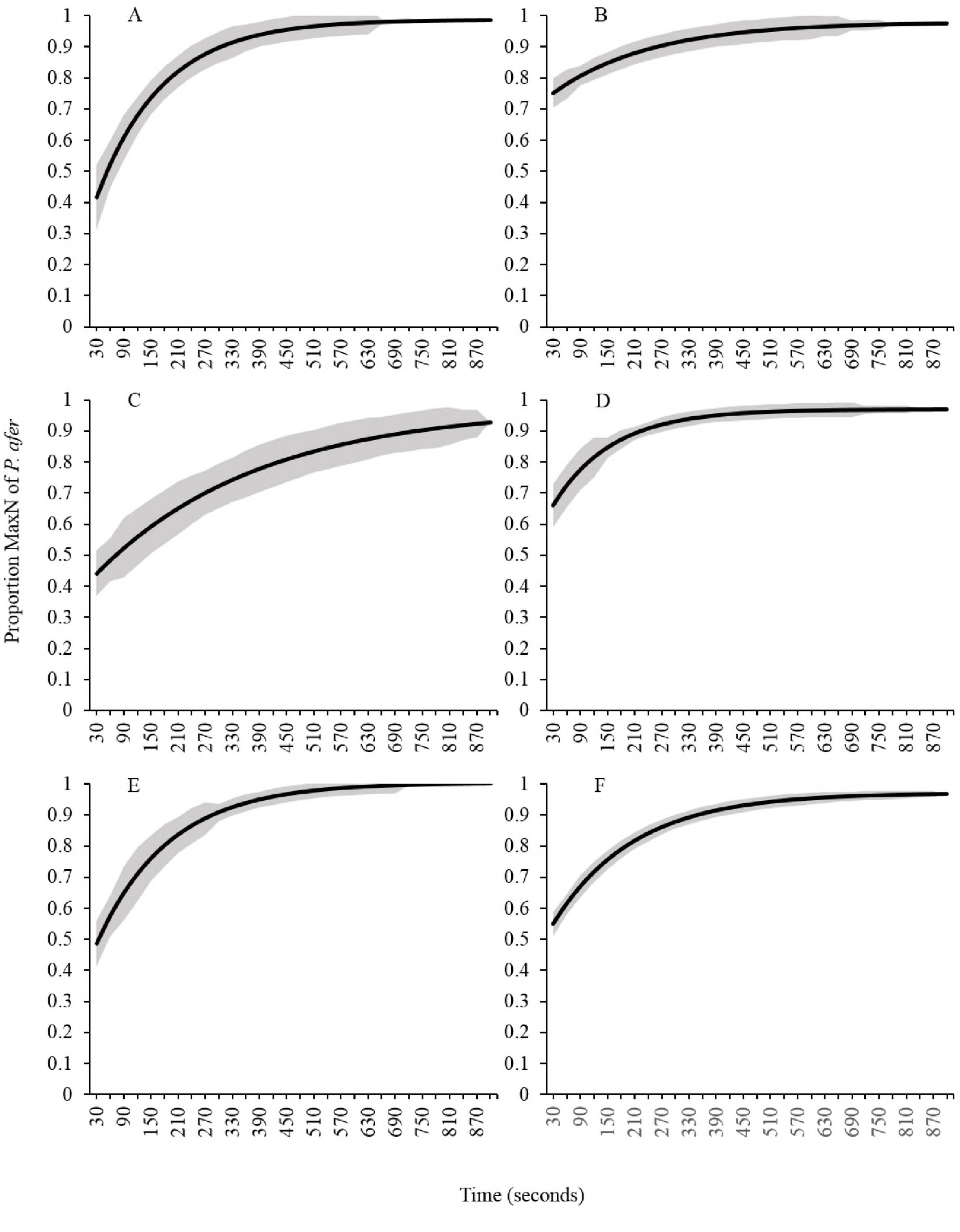

Figure 2.3. Accumulation curves over a 15-minute time interval based on MaxN values derived from videos for all five camera placements: A) inflow, B) woody debris, C) middle, D) fern root, E) outflow and F) all camera placements for ten pools found in headwater tributaries of the Swartkops River, South Africa. Grey shading indicates the upper and lower standard error values of the MaxN values.

2.3.4 Method comparisons

Absolute density estimates from snorkel surveys for *P. afer* in each pool and their relative abundance density from video-derived MaxN of the middle camera in each pool (Fig. 2.4.) were positively correlated (*Pearson's*; R = 0.84, d.f. = 8, $p < 0.01$), however, the MaxN derived from UWVA was significantly lower than the snorkel-survey density estimates (*t-test;* d.f. = 18, t =3.53, $p < 0.05$) with a regression slope of 0.607 (95% confidence interval (C.I.) 0.397-0.816 MaxN for *P. afer*). The resultant regression slope differed significantly from a 1:1 line (*ANCOVA;* d.f. = 16, t = 2.81, $p < 0.05$).

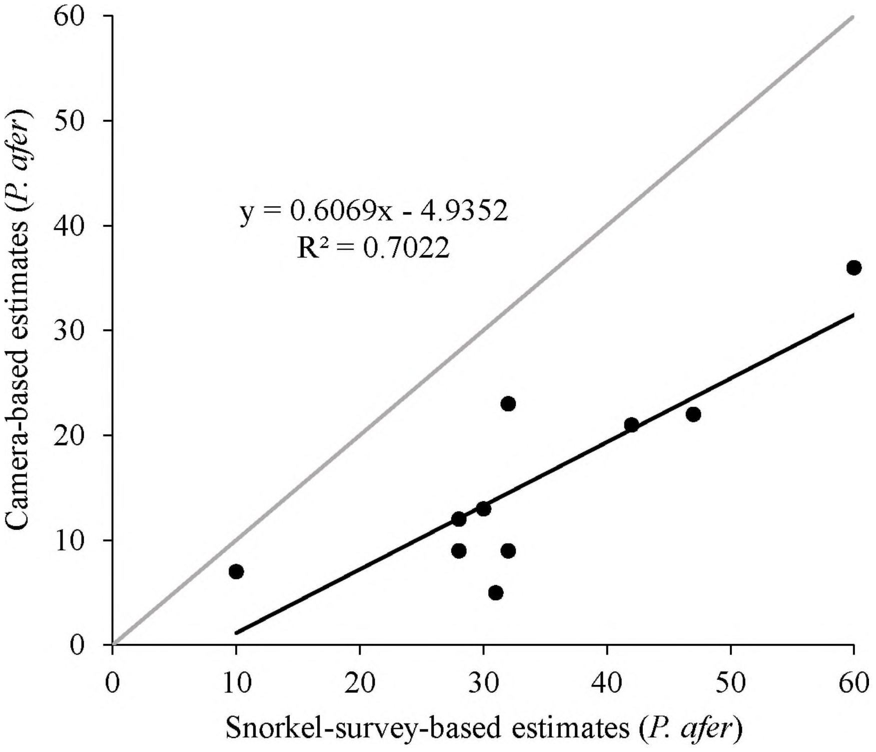

Figure 2.4. Snorkel and video abundance estimates derived from ten pools within headwater tributaries of the Swartkops River, Eastern Cape, South Africa. A 1:1 relationship is indicated by a grey line.

Photograph and video estimates for *P. afer* derived from the maximum MaxN estimates from the middle camera in each pool normalised by pool area, were both significantly different to the snorkel survey estimates (*t-test;* d.f. = 8, t = 5.407, $p < 0.001$; *t-test;* d.f. = 8, t = 4.217, $p < 0.01$), respectively (Fig. 2.5.). The gradient for the regression slopes were 0.485 (95% C.I.:

0.327 - 0.644 fish/m^2 and 0.414 (95% C.I.: 0.255 - 0.572 fish/m^2) for videos and still photographs, respectively. These differed significantly from a 1:1 relationship (*ANCOVA;* d.f. = 24, t = 4.742, p < 0.001) demonstrating that the two methods do not predict similar abundance estimates as snorkel surveys when only the middle camera is used. A possible solution to derive similar estimates would be to make use of multiple camera placements within the pool.

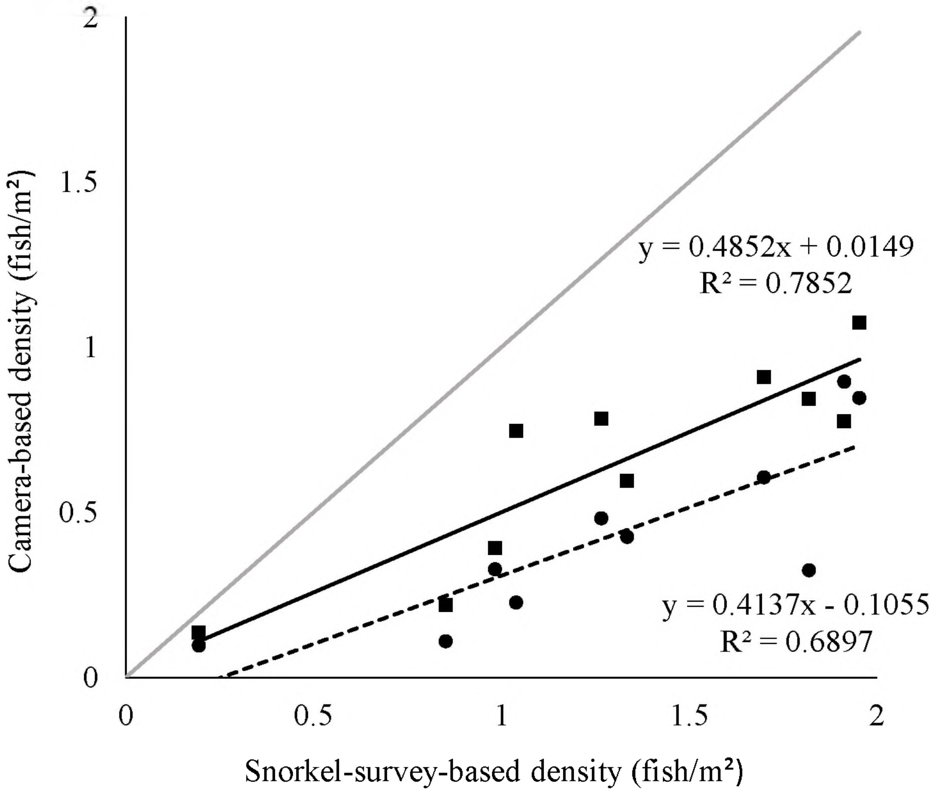

Figure 2.5. Maximum MaxN density estimates of *P. afer* derived from video (squares) and still photographs (circles) from the middle camera in each pool and snorkel-survey estimates normalised by the area of each pool for ten pools in two headwater tributaries of the Swartkops River, Eastern Cape, South Africa. The grey line indicates a 1:1 relationship.

Video and still photo density estimates for *P. afer* derived from the maximum MaxN estimates from all cameras in the pool, normalised by pool area (Fig. 2.6.), were not significantly different to the snorkel-survey estimates (*t-test;* d.f. = 18, t = 1.12, p = 0.28). The gradient for the regression slopes were 1.471 (95% C.I.: 1.165 – 1.779 fish/m^2 and 1.197 (95% C.I.: 0.890 – 1.504 fish/m^2) for video and still photographs, respectively. These did not differ significantly from a 1:1 relationship (*ANCOVA;* d.f. = 24, t = -2.24, p = 0.084). This demonstrated that video and photo derived density estimates were similar to snorkel-survey-based estimates when five simultaneous camera deployments within the pool were used rather than just the middle camera.

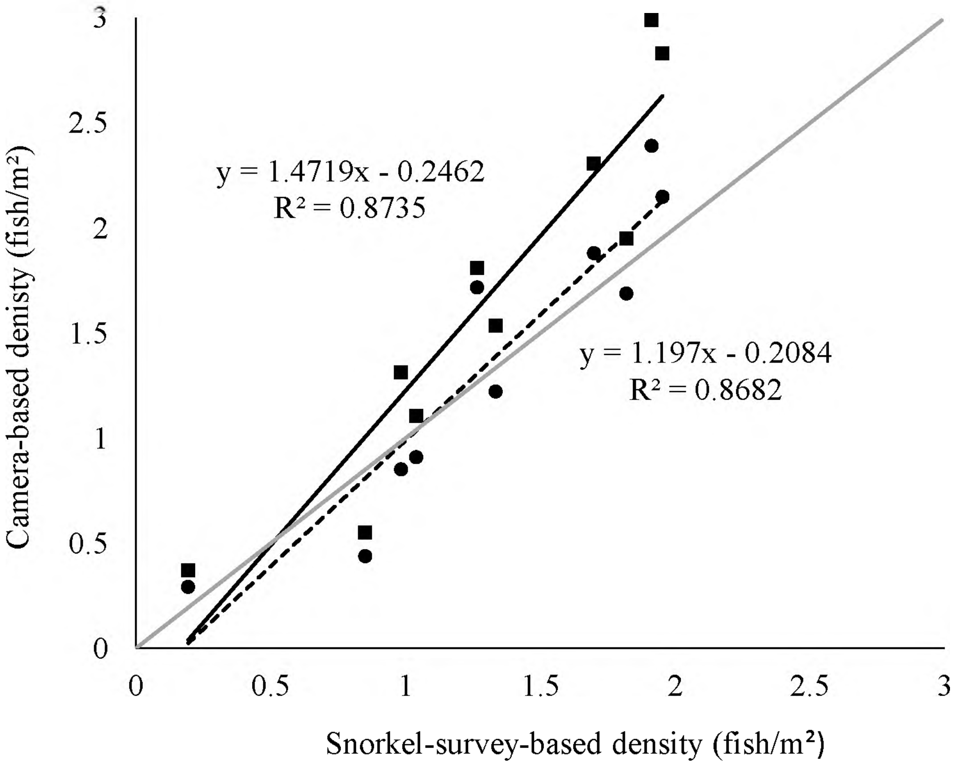

Figure 2.6. Maximum MaxN density estimates of *P. afer* derived from videos (squares) and still photographs (circles) from the middle camera in each pool and snorkel-survey estimates normalised by the area of each pool for ten pools in two headwater tributaries of the Swartkops River, Eastern Cape, South Africa. The grey line indicates a 1:1 relationship.

2.3.5 Multiple camera placements

A Shapiro-Wilk test for normality confirmed that the mMaxN density estimates (for videos) derived from cameras placed in different positions (Fig. 2.7.), were normally distributed. A repeated measure ANOVA (RMANOVA) showed a significant difference in the mMaxN density estimates from the respective camera placements in each pool (*ANOVA; F* (4, 45) = 6.24, $p < 0.001$). There was also a significant difference in mMaxN density estimates across the 10 pools (*ANOVA;* F (9, 40) = 3.57, $p < 0.001$).

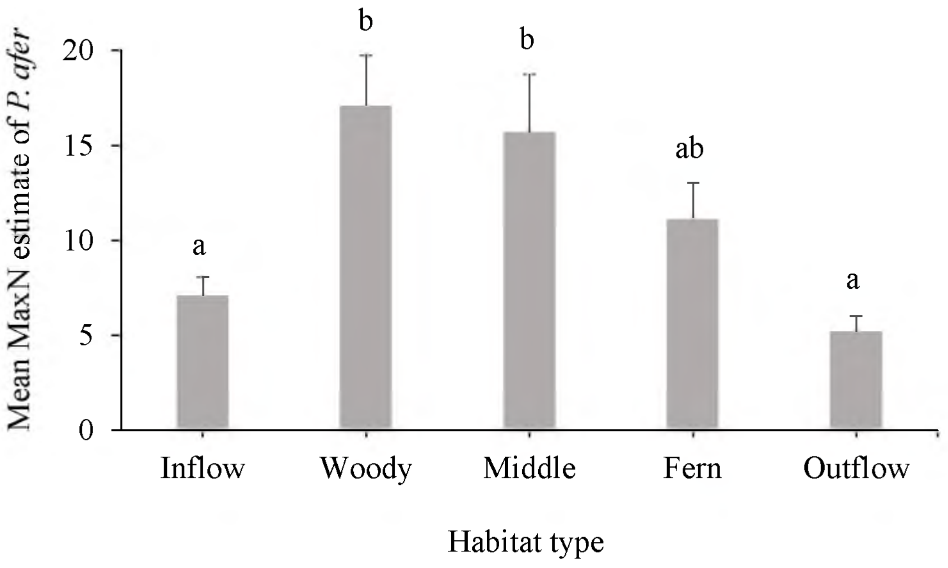

Figure 2.7. Mean MaxN (mMaxN) counts of *Pseudobarbus afer* taken from UWVA, grouped by habitat for ten pools in the Fernkloof tributary (lower-case letters that are the same indicate that there is no significant difference between mMaxN and lower-case letters that are different indicate where significant differences are found based on an ANOVA).

2.4 Discussion

In this chapter it was found that MaxN estimates of *P. afer* derived from action cameras were higher than those derived from snorkel-survey-based estimates. Furthermore, MaxN estimates derived from videos were found to be higher than those derived from still photographs in the ten pools sampled.

Prior to the recent advances in UWVA, snorkel surveys were conducted as a baseline method for detecting absolute abundances in streams (O' Neal, 2007). Pools in both the Blindekloof and Fernkloof tributaries were found to be appropriate for snorkel surveys as well as the use of action cameras for UWVA, for both videos and still photographs. There is a wide range in the number of fish detected in snorkel surveys across pools even after normalisation and this can be attributed to the difference in size between pools. Following normalisation of snorkel estimates by pool area, the differences in snorkel-derived estimates were reduced.

Accumulation curves showed that a minimum filming time of four minutes was sufficient to detect a proportion of 0.9 (out of the total mMaxN) of *P. afer* in one of the camera positions; woody debris. The longest time taken to reach a proportion of 0.9 cumulative mMaxN was 12.5 minutes for the middle habitat. The deployment time of 15 minutes was, therefore, found to be appropriate and was similar to or shorter than, the amount of time taken to sample in other freshwater studies (Ellender *et al.*, 2012; Weyl *et al.*, 2013) and a filming time of at least 12.5 minutes after an acclimation period is suggested.

Comparison between snorkel estimates and MaxN estimates from the middle camera found the snorkel estimates were considerably higher than MaxN estimates from only the middle camera and that the slope of the video vs snorkel regression line was significantly different to the 1:1 line. This indicated that the use of only one camera in a pool was insufficient to estimate the population size of *P. afer* in the pool. This finding showed that the electrofishing estimates derived by Ellender *et al.* (2012) may have underestimated the total number of *P. afer* individuals within the pools surveyed.

When MaxNs from all five cameras within a pool were summed, still photographs and video density estimates for *P. afer* were both found to have strong correlations with densities derived from snorkel surveys, indicating that they were both appropriate methods for determining fish abundances. When the slopes of both were compared with a 1:1 relationship with snorkelling densities, it was found that there was no significant difference between camera-based fish density estimates and those from snorkel surveys. This supported that either of the camera-based methods could be used in place of snorkel surveys. Five cameras were used for this study. Future work should evaluate whether this is the optimum number of cameras for fish density estimates. Two cameras, covering the inflow and outflow of the pools, recorded very low densities and it is possible that these could be excluded from the cumulative pool fish density estimate without significantly affecting the outcome.

The use of action cameras allows the observer bias associated with snorkelers or divers to be addressed. Previous studies using snorkelers or scuba divers have shown that fish behaviour may be influenced by observers (Cappo *et al.*, 2003; Jordan *et al.*, 2008, Assis *et al.*, 2013), potentially skewing fish counts. Furthermore, snorkel surveys may be better suited to waterbodies that are not too deep or too big, as this may also result in skewed counts (O'Neal, 2007). However, as an added benefit, snorkel surveys require minimal equipment and can be used to sample remote areas.

This chapter initially focussed on MaxN estimates derived from the middle camera as per recommendation from Ellender *et al.,* (2012). The camera placed in the middle of the pool, yielded the highest MaxN estimates and was, therefore, thought to be the best placement for cameras. However, the highest number of fishes also resulted in the highest standard deviation and therefore, the middle MaxN counts varied the greatest among the five cameras in each pool. When other camera placements were used, it was found that large numbers of *P. afer* were also detected in other habitats. For example, the camera placements in this study varied between five different habitat types and the MaxN counts from the woody debris were similar to than those from the middle. This may not be the case for all fishes as some species may be cryptic for example, in a study done by Weyl *et al.,* (2013), detection rates by UWVA varied based on species. Fish that were more cryptic were most likely detected in electrofishing or snorkelling, but not by UWVA. For camera-based sampling to be effective, it is recommended from this study that multiple camera placements (a minimum of three) within a pool be used in preference to a single camera. For future studies, researchers should consider the fact the size of each pool will influence the number of cameras required to obtain the highest counts of fish.

This chapter showed that a 15-minute filming time is adequate to detect 0.9 of the cumulative mMaxN estimate of the fish abundance in the pool. Furthermore, the use of estimates derived from videos and photographs were appropriate alternatives to the use of classic methods, such as snorkel surveys especially when multiple cameras were used. Therefore, cameras were used as the main technique for recording fish abundance data in the remainder of the studies in this thesis and a filming period more than or equal to 15 minutes will be used in conjunction with a 3-minute acclimation time.

Furthermore, estimates from still photographs and videos were correlated to determine whether there was a significant difference in MaxN estimates derived from both methods. The use of still photographs instead of videos may drastically reduce analysis time and provide the researcher with the potential to sample more sites in less time. Still photographs can provide a quantitative count on how many fish are present in a certain area while videos can be used for behavioural inferences. Recommendations for future studies should include testing whether the three-minute acclimation time used in this study is sufficient and determining the number of cameras per pool required to cover all habitats available in the pool and to detect the maximum number of fish

CHAPTER 3

HABITAT USE BY *PSEUDOBARBUS AFER* (PETERS, 1864) AND *SANDELIA CAPENSIS* (CUVIER, 1829)

3.1 Introduction

Habitat requirements of fish species depend on their specific needs (Orpwood *et al.*, 2008). The need to find food or shelter could favour learned behaviours such as foraging in habitats where food is abundant or present or finding shelter when predators are detected (Odling-Smee *et al.*, 2011). Similarly, habitat use by fishes will also be dependent on their stage of life cycle (Schlosser, 1991). Stream fishes increase in size throughout their life cycle and, associated with this change in size, is the change in habitat-use patterns (Schlosser, 1991). Habitat requirements through all phases of the life cycle of fishes were poorly understood (Fausch, 2002) and this may have presented a challenge for stream-fish ecologists to determine the link between fishes and their habitat use and preference (Schlosser, 1991). However, recently more emphasis has been placed on studies to help researchers better understand habitat requirements (Rice, 2005).

Schlosser (1991) created a model to illustrate habitat use and preference by fish species throughout different stages in their life cycle (Fig. 3.1.). Since it is evident that movement between habitat types is based on each stage of the life cycle and the requirements of the fish species, an emphasis needs to be placed on understanding habitat use not only from the fish's perspective, but also at a habitat level, monitoring each individual habitat. Fish species may often utilise a mosaic of habitats for feeding and refuge causing a wide array of habitat associations. Environments that are heterogeneous are favourable because of the combination of resources that each habitat provides opposed to relatively few provided in homogenous environments (Schlosser, 1991). Due to trophic niches frequently overlapping (Wilbur, 1980), heterogeneous environments are required to allow fish species to coexist.

Studies of fish's habitat use in headwater streams and larger rivers have shown that there are associations between structural characteristics of each habitat and the occurrence of fish species (Schlosser, 1991), for example habitat structure/complexity has been shown to influence the service that it provides to fish species (Tews *et al.*, 2004). Habitat complexity created by certain habitat types provide refuge for escape from predators, thereby increasing survival rates of the prey fish species and reducing predator efficiency (Crowder & Copper 1979; Savino & Stein 1982); for example, submerged macrophytes (Diehl, 1988).

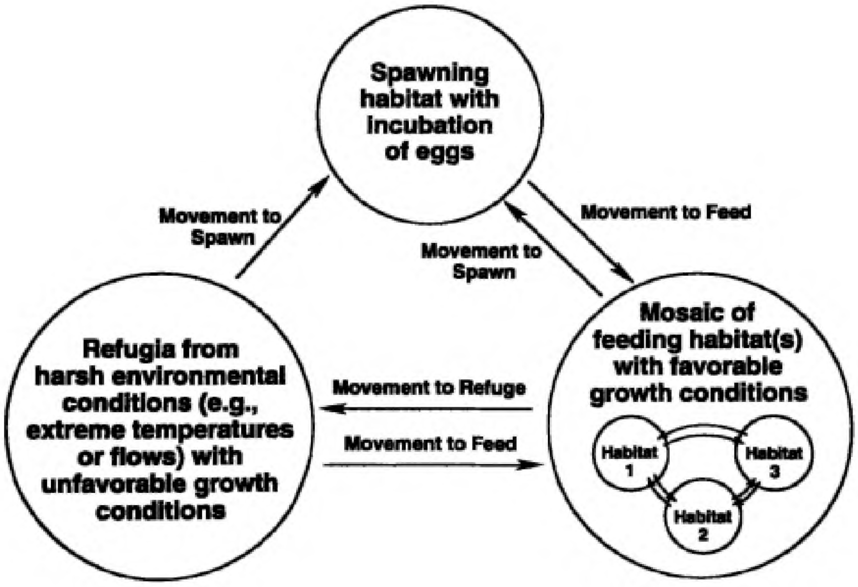

Figure 3.1. Basic life cycle of a stream fish showing different habitat requirements at different stages of the life cycle (Schlosser, 1995).

Alexander *et al.* (2015) showed that as habitat complexity decreased, a significantly higher biomass of fish prey was consumed by predators. For their study, largemouth bass *Micropterus salmoides* (Lacépède, 1802) were set up in experimental tanks with artificial habitats at varying complexity levels (high, medium, low and zero). Female guppies *Poecilia reticulata* Peters, 1859 were used as prey and were introduced at varying prey densities into tanks containing *M. salmoides*. It was found that decreasing habitat complexity resulted in a significant increase in prey consumption. Similarly, Orpwood *et al.* (2008) evaluated whether the effects of predation on schooling behaviour were dependent on habitat complexity. In this study, the response of wild European minnow *Phoxinus phoxinus* (Linnaeus, 1758) to the presence of predatory pike *Esox lucius* (Linnaeus, 1758) were tested in tanks with varying degrees of habitat complexity. Schooling behaviour of *P. phoxinus* was significantly more evident with the presence of a predator and in habitats that were regarded as simple.

These studies showed that when prey fishes were in the presence of predator fishes, prey fishes utilised more complex habitats for refuge, thereby increasing their potential survival. Habitats that are viewed as complex may be termed critical habitats as they are able to provide a wide array of services to respective fishes. Critical habitats are usually habitats that have a positive relationship with population size (Rosenfeld & Hatfield, 2006) in addition to areas that are essential to their survival.

The monitoring of habitat utilisation alone does not provide sufficient information on the critical habitat requirements for a particular species. The behaviour of the fish needs to be evaluated to determine why each habitat is being used. However, "*the variety of behaviours found in the animal kingdom are so vast and that an understanding of various behavioural syndromes will only be possible once one understands why the behavioural trait originated*" (Tinbergen, 1963). Understanding the cause of the behaviour in question (causation), its survival value (function), how it developed (ontogeny) and how it evolved (evolution) will aid in studies investigating why some individuals behave in a specific way (Tinbergen, 1963).

Orpwood *et al.* (2008) addressed the behavioural syndromes as posed by Tinbergen (1963), in their study of fish responses to habitat complexity. The causation of schooling behaviour was attributed to the presence of the predatory species and the lack of a complex habitat for refuge. Orpwood *et al.* (2008) concluded that the function of schooling behaviour was to reduce the risk of an individual being predated upon by aggregating in a group, the ontogeny of schooling was a learned behaviour with the evolutionary benefit of this behaviour being passed from generation to generation. Furthermore, to assess behaviour of in-stream fishes, the shy-bold continuum (Wilson *et al.*, 1994) can be used to measure the fish's propensity to take risks, such as spend time in an open habitat or inspection of an object that has been introduced into a waterbody.

Understanding the behaviour and habitat utilisation of a fish species will help inform the conservation steps needed to protect species from constant environmental threats; this is particularly true for imperilled species. A constantly changing global environment and human-mediated impacts, for example, pollution, over abstraction of water or flow modification (Stiassny, 1999; Cowx, 2002; Dudgeon *et al.*, 2006; Strayer & Dudgeon, 2010), are placing freshwater fishes and their habitats at risk. One such area facing these drastic changes is the Cape Fold Ecoregion (CFE) in South Africa. Threats, such as an increased rate of the introduction of non-native invasive species into freshwater systems, are placing endemic species further at risk for population alterations (Dudgeon, 2006). Through the alteration of these waterbodies, habitat destruction or degradation is inevitable and critical habitats are being placed at risk. Both observations on habitat utilisation and inferences about behaviour can be achieved using videos or photographs. As seen in Chapter 2, estimates derived from snorkel surveys were strongly correlated to estimates derived from MaxN counts from videos. Furthermore, photographs can be used in place of videos to drastically reduce analysis time.

There are drawbacks and advantages to both methods. Two major drawbacks of using videos are that the storage space required may be excessive and the viewing time required during analysis is lengthy (Cappo *et al.*, 2003; Ebner *et al.*, 2009; Struthers *et al.*, 2015). A 15-minute video can take from 45 minutes to an hour to analyse while photographs require less storage space and less time to analyse while a set of photographs taken for the same period can take approximately 25 to 35 minutes. However, videos are more suitable for making inferences about behaviour than photographs (Willis & Babcock, 2000; Willis *et al.*, 2000; Pratt *et al.*, 2005; Ebner *et al.*, 2009) while quantitative counts of fish from photographs may be easier, they may also provide lower estimates than videos.

This chapter will assess the habitat utilisation and preference of the imperilled Eastern Cape redfin *Pseudobarbus afer* (Peters, 1864) and the Cape kurper *Sandelia capensis* (Cuvier, 1829) in the Fernkloof tributary of the Swartkops River, Eastern Cape, South Africa. The present study aimed to build on findings from Chapter 2 and was completed as a proof of methods to be used in Chapter 4 and as a baseline for *P. afer* and *S. capensis* habitat utilisation. Therefore, the objectives were to evaluate whether estimates derived from videos and still photographs are strongly correlated and, if so, still photographs could be used in place of videos to reduce analysis time. In this study, the habitat utilisation and preference of, *P. afer* and *S. capensis* were evaluated by: (1) assessing whether still photographs can be used in place of videos to reduce analysis time; (2) quantitatively assessing habitat utilisation by *P. afer* and *S. capensis* with the use of *in situ* action cameras to gain a better understanding of which habitats are being utilised and why; and, (3) attempting to qualitatively describe how they utilise instream habitats by looking at the function and causation of the behaviour displayed in the respective habitats.

Since very little work has been done on the habitat preference of these two species, it was difficult to make predictions in the beginning of the study. Through literature review and trial runs using action cameras, it was hypothesised that *P. afer* would most likely be associated with open habitat areas and that *S. capensis* would most likely be less frequently spotted on the cameras and be associated with complex habitat types.

3.2 Methods and materials

3.2.1 Study site

This study evaluated habitat use and behaviour of *P. afer* and *S. capensis* in four pools of the lower reaches of the Fernkloof headwater tributary of the Swartkops River, Eastern Cape, South Africa (33°43’ S, 25°17’ E; Fig. 3.2.). The Fernkloof tributary lies entirely within the Groendal Nature Reserve and has been minimally impacted by human activities. At the time of the survey, the flow of water was low between pools. The substrate within each pool is characterised by boulders which were > 256 mm in diameter (found in the inflow and outflow portion of each pool), gravel which were 10 – 64 mm in diameter and cobble stones which were 65-256 mm in diameter (in all other portions of the pool). Other fish present within the pools at the time of the survey were the introduced banded tilapia *Tilapia sparrminii* Smith, 1840.

3.2.2 Site selection

Data for this chapter were collected *in situ* in the Fernkloof tributary of the Swartkops River over the week of 12th – 18th of February 2017. Four pools were chosen in total (GPS coordinates were recorded for each pool) and each pool was only sampled once. The four pools were selected based on the presence of the target species, *P. afer* and *S. capensis*, and the obligatory presence of a suitable variety of habitats including inflow riffle, outflow riffle, deep middle section, woody debris and fern root i.e. the fern is found on the side of the pool with their roots extending into the pool.

Water physico-chemical parameters were measured using a HANNA HI98129 combo probe reading pH, temperature, and electrical conductivity (HANNA Instruments, Inc., United States of America.). A portable HANNA HI 98703 turbidity meter (HANNA Instruments, Inc., United States of America.) was used to measure turbidity. Three measurements of each parameter were recorded in each pool and averaged for each pool (Table 3.1).

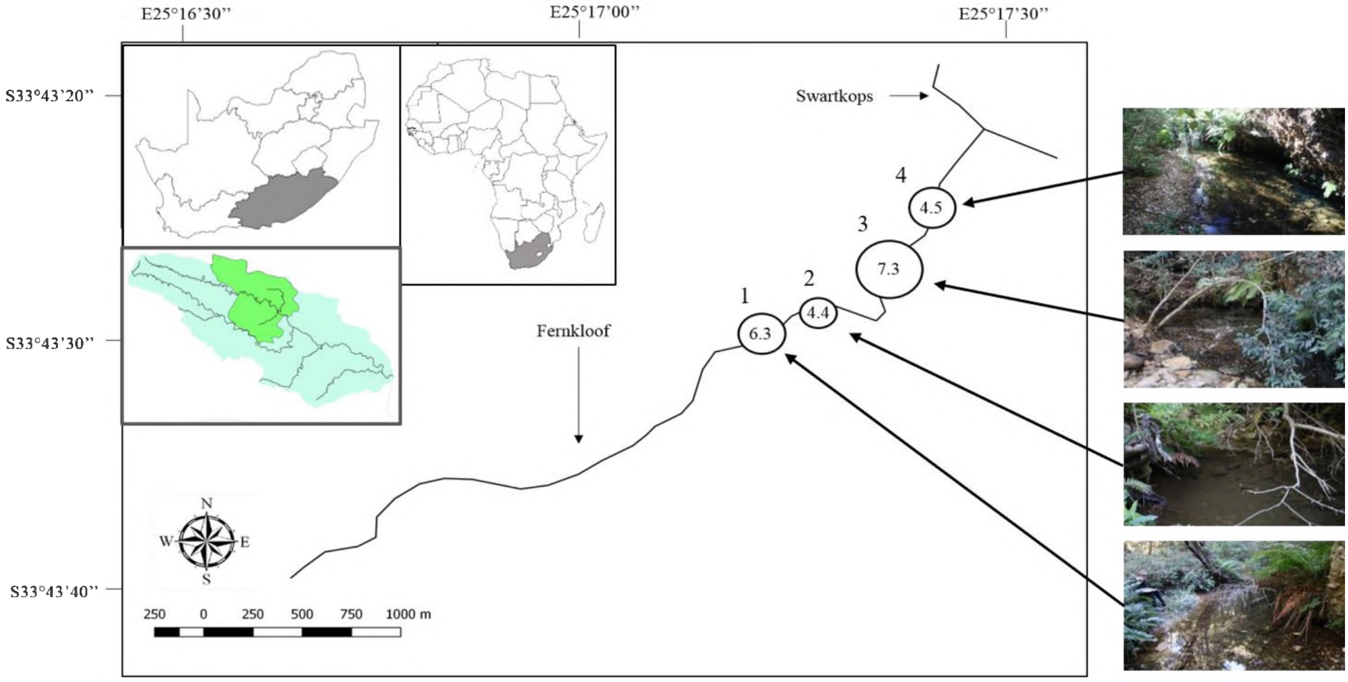

Figure 3.2. Locations of the Fernkloof tributary in relation to the Swartkops River in the Eastern Cape, South Africa as well as the location of the study site in relation to the Swartkops drainage basin (blue) and the Groendal Nature Reserve (green). Pools are indicated by white circles and are sized according to the size of each pool showing the volume of each pool in m³ (Table 3.1). Numbers above pools indicate site numbers and correspond with pools used in Chapter 2. Photo inserts show what each pool looked like.

3.2.3 Habitat mapping

Extensive habitat maps were drawn in the field for each of the four pools. A schematic diagram is represented in Fig 3.3. In addition, detailed measurements were recorded to prepare a detailed depth contour map for each pool. A 50-meter measuring tape was used to measure the length of each pool and established as a centre line for the pool. Thereafter, a three-meter measuring tape was used to measure the distance from the centre line to each bank, at every metre perpendicular to the centre line (Fig 3.3; line B). Along each width transect, the depth was measured every 25 cm from the centre line to the wetted edge using a graduated depth stick. Inflow, outflow and habitat types were noted on the annotated maps. Depth contour maps were created using the contourplot function in R 3.41 statistical software (R Development Core Team. 2016) through RStudio 0.99.902 (Rstudio, Inc.). Habitat maps were used to calculate volume of each pool.

Table 3.1. Summary statistics produced from habitat measurements in the four pools found in the Fernkloof tributary in the Eastern Cape, South Africa. Physico-chemical properties are shown as the mean reading (from three readings) per pool ± SD.

Pool	Location	Length (m)	Mean width (m)	Mean depth (m)	Volume (m^3)	Water temperature (°C)	Conductivity (µs/cm)	Turbidity (NTU)	pH
1	33°43'24.64"S, 25°17'7.80"E	13 ± 0.4	2.6 ± 0.4	0.29 ± 0.02	4.52	19.5 ± 0.3	227 ± 1	0.52 ± 0.14	4.66 ± 0.35
2	33°43'26.65"S, 25°17'5.28"E	17 ± 0.5	3.0 ± 0.4	0.27 ± 0.02	7.25	18.8 ± 0.3	227 ± 1	0.24 ± 0.27	4.95 ± 0.15
3	33°43'27.26"S, 25°17'3.72"E	10 ± 0.3	3.5 ± 0.9	0.23 ± 0.01	4.37	19.7 ± 0.4	234 ± 1	0.30 ± 0. 02	5.49 ± 0.24
4	33°43'27.45"S, 25°17'3.33"E	10 ± 0.3	3.1 ± 0.7	0.47 ± 0.03	6.30	20.2 ± 0.7	240 ± 1	0.31 ± 0.02	4.87 ± 0.50

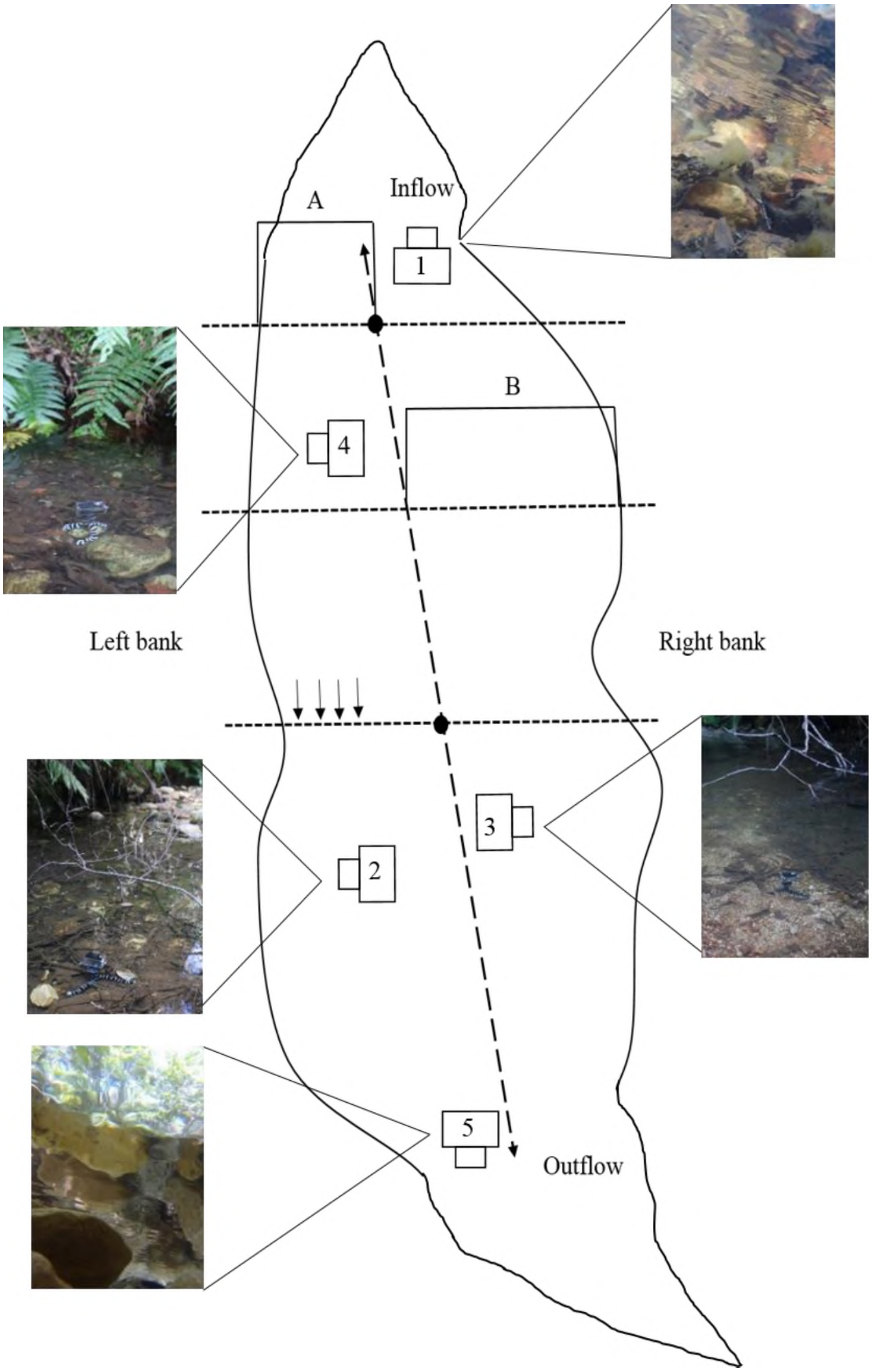

Figure 3.3. Schematic diagram depicting the method used to measure each pool for the habitat map and depth contour plot. A and B represent the measurements from the left wetted bank to the centre and the right wetted bank to the centre respectively. Dashed line (−−) in the middle represents the length transect of the pool whilst the smaller dashed line represents an example of how width measurements were taken (---). Photographs are depictions of what each habitat (inflow, fern root, middle, woody debris and outflow) looked like and where cameras were placed (number 1 – 5 respectively).

3.2.4 Evaluating still photographs vs UWVA

Data collected for Chapter 2 were used here to assess whether photographs would yield similar estimates to videos to reduce analysis time. From the five cameras set up in each pool for ten pools (Chapter 2), a MaxN was determined for each camera. This yielded 50 MaxN estimates for both videos and photographs. To test for whether there was a significant difference in MaxN estimates derived from videos and still photographs, a Pearson correlation was used using the 'cor.test' function in R. Furthermore, an ANCOVA was used to test whether there was a significant difference in the gradient of the slope to a 1:1 relationship.

3.2.5 Fish data collection

Following analysis on still photo and video estimates derived from methods in Chapter 2, action cameras (GoPro Hero 3+, GoPro Inc., United States of America) were used to document the habitat utilisation of *P. afer* and *S. capensis* in four pools in the Fernkloof tributary of the Swartkops River. Five cameras were placed on gorilla tripods during daylight hours facing five different habitat types (inflow, woody debris, middle, fern root and outflow) in each pool; (see Fig. 3.3). The cameras were placed approximately 1.5 m away from each habitat type such that each camera field of view did not overlap with that of any other camera. The area covered by each camera was not equivalent for all habitats due to varying sizes of habitat patches and varying fields of view for example, the inflow and outflow had a limited field of view while the camera in the middle was able to detect fish swimming meters away from the camera. Action cameras were turned on, placed in position, after which the observer left the pool and waited out of sight. In all four pools, cameras were set to take photographs (at 12-megapixel resolution) every five seconds to battery extinction.

To evaluate the behaviour of *P. afer* and *S. capensis* in the respective habitats, cameras in one pool were set to concurrently film video (at a frame rate of 720 fps) and take still photographs every five seconds. Due to differential battery life, a standard set of 750 photographs (the number of photographs taken prior to the battery extinction of the first camera) or 62.5 minutes of filming time were viewed for each camera.

Still photographs were viewed in Windows Photo Viewer (Microsoft Inc.). The number of *P. afer* and *S. capensis* in each photo was recorded (N). The maximum number of fish seen in each 30 second time interval (6 photographs) was recorded as the MaxN for that time interval, which was used to provide a quantitative assessment of the number of *P. afer* and *S. capensis* in each habitat within each pool for each time interval.

3.2.6 Habitat preference

Proportional occupancy was used instead of raw MaxN values to account for the number of fish present in each pool. To calculate a proportional occupancy for each time interval, a ΣMaxN was calculated over all habitats in each pool for each time interval and habitat maximum MaxN for each time interval was divided by the corresponding ΣMaxN. This resulted in 125 proportional occupancies for each habitat, in each pool, for each species. Proportional occupancy for each habitat was checked for normality using the Shapiro-Wilk test. The data were not normally distributed. The data were transformed using the arcsine-square root transformation ($\sqrt{\arcsin(x)}$) but was still not normally distributed, therefore the raw data were used.

A two-way analysis of variance was performed to test whether there was a significant difference in proportional occupancy between pools, habitat type, and/or the interaction between habitat type and pool. Finding that there was no significant relationship for pools but a significant result for the pool-habitat type interaction, a one-way ANOVA was used to determine whether there was a significant difference in proportional occupancy by habitat type. The ANOVA was used in preference to the non-parametric Kruskal-Wallis test due to the large sample size (van Hecke, 2012).

The ANOVAs were performed for each species independently to test whether there was a significant difference in the proportional occupancies of *P. afer* and *S. capensis* across habitats. A post-hoc Tukey test was conducted to determine which of the habitat type pairs were significantly different from each other.

3.2.7 Behaviour

Videos from Pool 4 were viewed in Windows Media Player (Microsoft Inc.). This allowed for observations on the behaviour of fish in each habitat to evaluate the purpose for which the habitat was being used. Habitat use was qualified using pre-defined behavioural traits: refuge, schooling, feeding and resting.

The behavioural trait feeding was used if a fish was observed to be ‘picking’ an object off the substrate and the behavioural trait resting was used if a fish appeared to be at rest and was neither schooling, eating nor using the habitat for refuge. The behavioural trait refuge was assigned if a fish was seen to be using structure that provided the fish with cover whereas schooling was assigned if there was no apparent association with any structure and the fish were generally found in the middle area. A single sampler viewed and scored the behaviour to reduce observer bias and conflicting opinions of fish behaviour.

Each behaviour was scored in accordance to its frequency of occurrence in each habitat for example, every time a behaviour was observed, it was given a score of one and the second time it was observed another score of one was added. A score of zero was given if a specific behaviour was not observed in a habitat. Behaviour was analysed across habitats types using a Chi-Square test of independence to test whether there was a significant difference in behaviours observed.

Behavioural observations of *S. capensis* were analysed using a Fisher's Exact test because of the small sample size and the minimum expected value being low when a Chi-Square test was used. The inflow was not included in analyses as no *S. capensis* were observed in this habitat. For analyses, a 2 x 2 contingency table was used grouping counts from foraging and refuge for woody and fern habitats and grouping counts from resting and schooling for the middle and outflow.

All data analyses were conducted using R 3.4.1 statistical software (R Development Core Team. 2016) through RStudio 0.99.902 (RStudio, Inc.) and MS Excel 2013 (Microsoft). All statistical analyses used $p = 0.05$ to determine statistical significance.

3.3 Results

3.3.1 Habitat description

During the surveys, pool water was very clear (mean ± standard deviation: 0.34 ± 0.13 NTU), with an average water temperature (19.5 ± 0.7°C), a slightly acidic pH (range: 4.32-5.64) and a conductivity of 232 ± 6 µS/cm. Physico-chemical properties are shown in Table 3.1. Pools 1, 2 and 4 each had a cliff face on one side with the deepest point along the cliff face. These pools became shallower towards the opposite bank (Fig. 3.4). Pool 3 was the exception being deepest at the centre of the pool and getting shallower towards each bank. Summary statistics from the habitat measurements and GPS locations are presented in Table 3.1.

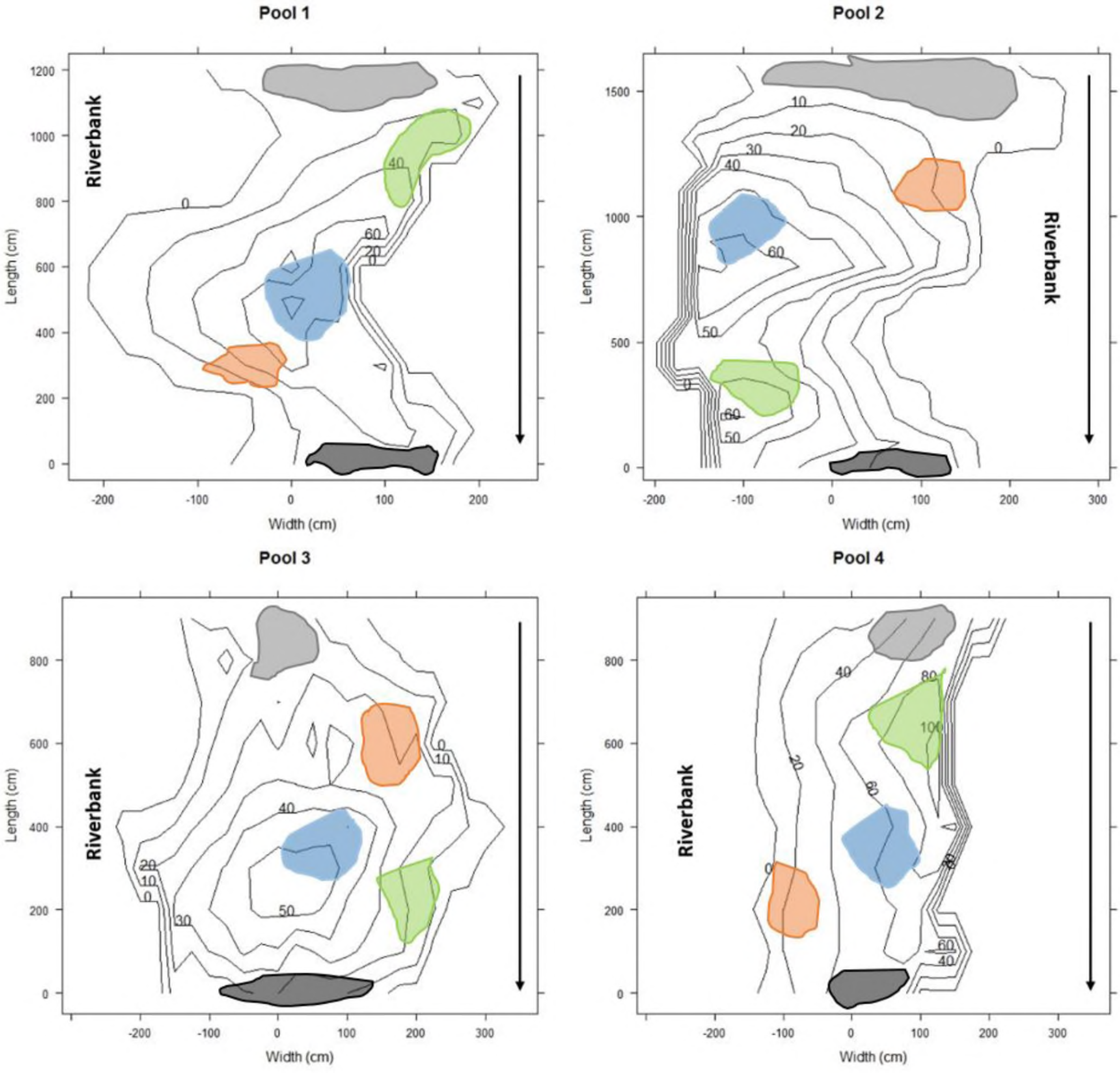

Figure 3.4. Contour plots showing length (cm), width (cm) and depth (cm) of four pools in the Fernkloof tributary of the Swartkops River, Eastern Cape. The arrows indicate the direction of flow of water from upstream to downstream and the top and bottom of each plot represent the head and tail of each pool respectively. Colours represent the relative camera placements throughout the four pools (dark grey = inflow; orange = woody debris; blue = middle; green = fern root; black = outflow).

3.3.2 Videos vs still photographs

Still photographs were used to quantify habitat preference of *P. afer* and *S. capensis* under the assumption that a fish would only be present in one habitat during a specific 30 second time interval due to non-overlapping fields of view for all the cameras. The relationship between videos and photographs derived from MaxN estimates is shown in Figure 3.5. A strong significant correlation was found between photo and video estimates (*Pearson's;* R = 0.862, n = 50, $p < 0.001$). An ANOVA showed that the both factors (pool and method) and the interaction term were significant, (*ANOVA;* F (1, 96) = 27.4, $p < 0.001$). The slope of the trend line was 1.108 (95% CI 0.976 – 1.240 *P. afer*) and was not significantly different to 1 (*t-test;* d.f. = 96, t =-1.286, $p = 0.202$) indicating that similar estimates are derived from videos and still photographs.

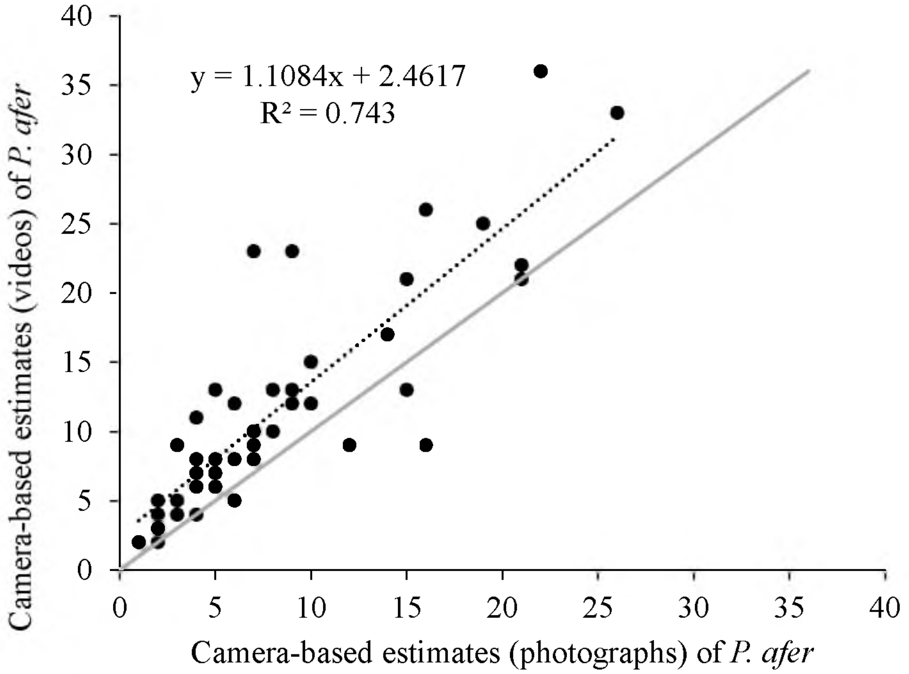

Figure 3.5. MaxN estimates derived from photographs and videos from 5 different camera placements in ten pools in two headwater tributaries of the Swartkops River, Eastern Cape, South Africa. A 1:1 line is depicted by a grey solid line.

3.3.3 Habitat use

The majority of the *P. afer* appeared to prefer the middle of the pool, followed by the woody debris and fern habitat (Fig. 3.6). Inflow and outflow had very low occupancy across all four pools (Fig. 3.6). The MaxN estimates of *P. afer* for the individual habitats were not normally distributed (*Shapiro-Wilk*; $p < 0.001$. Due to the large number of points in the dataset, it was decided to continue with the ANOVA analysis of the raw proportional occupancy data. The interaction term between pool and habitat were significantly different (*two-way ANOVA*; F (12, 2460) = 201.9, $p < 0.001$). There was no significant difference in proportional occupancy between pools (*two-way ANOVA*; F (3, 2460) = 0.00, $p = 1$). This showed that pool could be discounted as a factor. Proportional occupancy of *P. afer* across habitats was found to be significantly different (*ANOVA;* F (4, 2475) = 9384, $p < 0.001$).

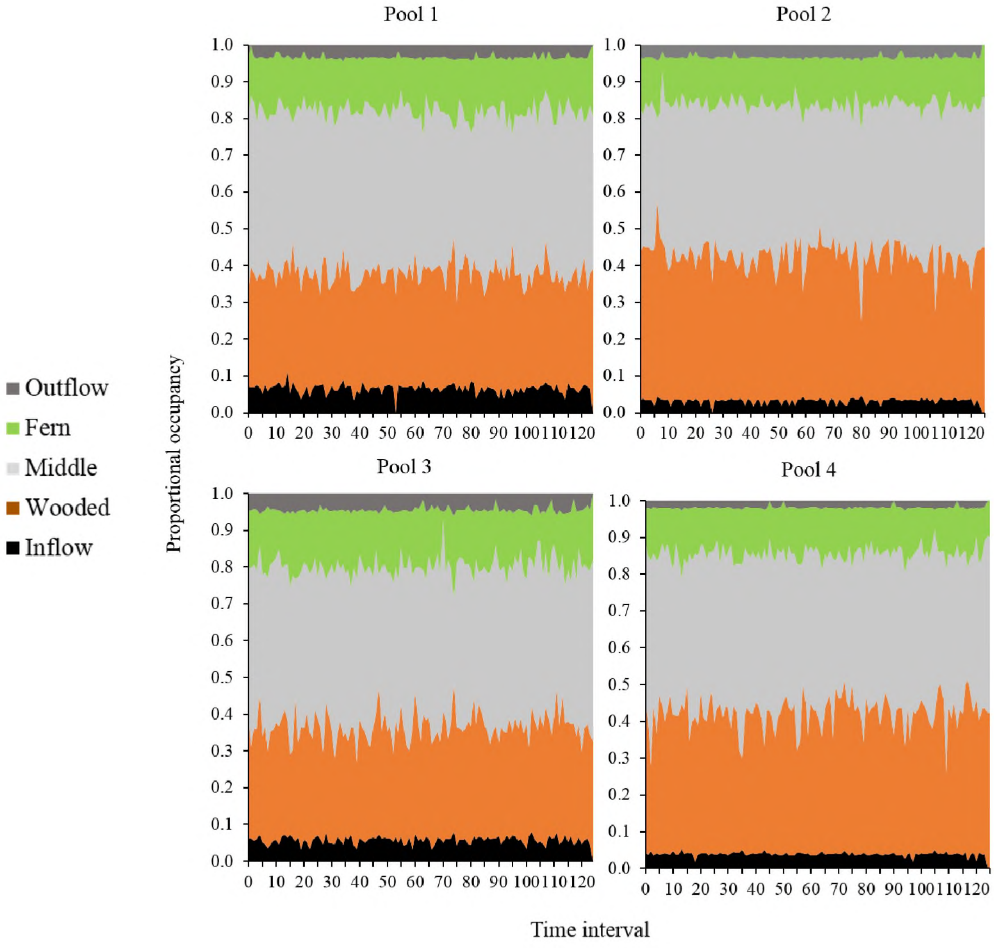

Figure 3.6. Proportion of *Pseudobarbus afer* detected in each habitat (inflow, fern, middle, woody and outflow) across four pools (1, 2, 3 & 4) in a headwater tributary of the Swartkops River, Eastern Cape, South Africa.

After proportional occupancy for *P. afer* was summed per habitat (all proportional occupancies were added together for the respective habitats over the four pools), it was found that there was a significant difference (*ANOVA*; F (4, 15) = 223.5, $p < 0.001$) in Σ proportions over the five habitats (Fig 3.7.).

There is a large difference in proportion occupancies across all five habitats with the middle habitat having the highest proportion occupancy (mean ± SD; 0.41 ± 0.11) and the inflow and outflow having comparatively low proportion occupancy ($p < 0.1$; Fig. 3.7.). A post-hoc Tukey test on proportional occupancy per habitat showed that all habitat interactions, except the one between the inflow and outflow, were significantly diffferent for *P. afer* ($p < 0.001$).

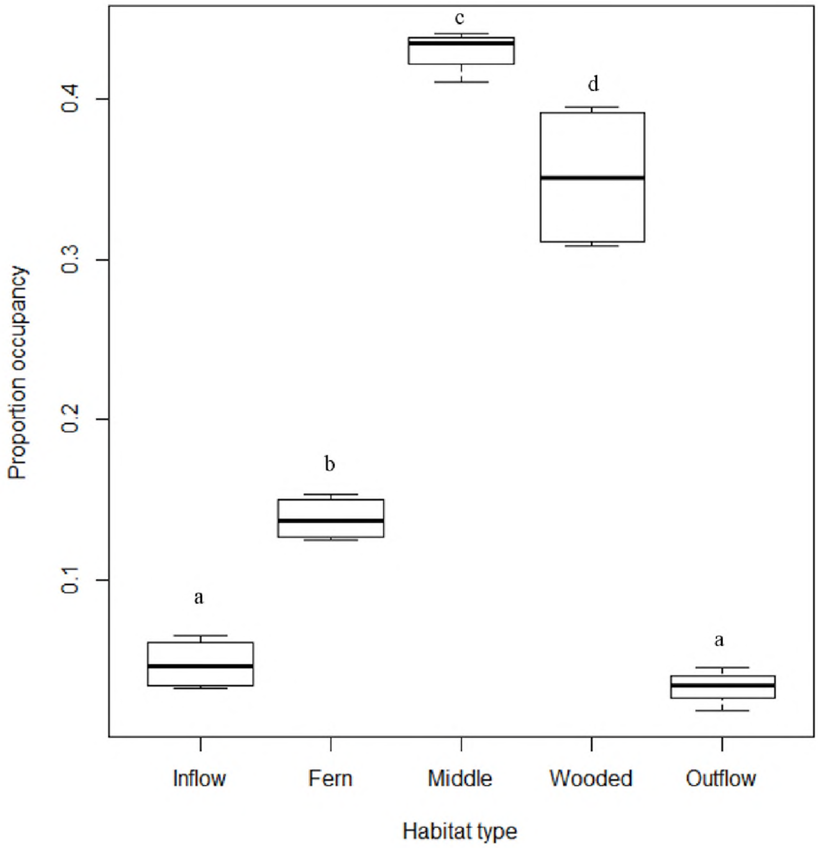

Figure 3.7. Mean proportional occupancy of *Pseudobarbus afer* in five habitat types within four pools of the Fernkloof headwater tributary of the Swartkops River, Eastern Cape, South Africa (lower case letters (a-d) represent where there were significant differences, tested with an ANOVA, in proportional occupancies between habitats).

Sandelia capensis was found to favour the woody debris structure over the other four habitats in the pools (Fig. 3.8) where a minimum of one *S. capensis* was recorded for approximately 58 minutes out of the total 62.5 minutes filming time. The exception was Pool 2 where the fern root area was favoured. There was low proportional occupancy of *S. capensis* in the outflow with only two pools having *S. capensis* in the outflow. No *S. capensis* were found in the inflow habitats in any of the pools. The proportional occupancy for the individual habitats were tested for normality using the Shapiro-Wilk test with all habitats returned significant results ($p < 0.001$).

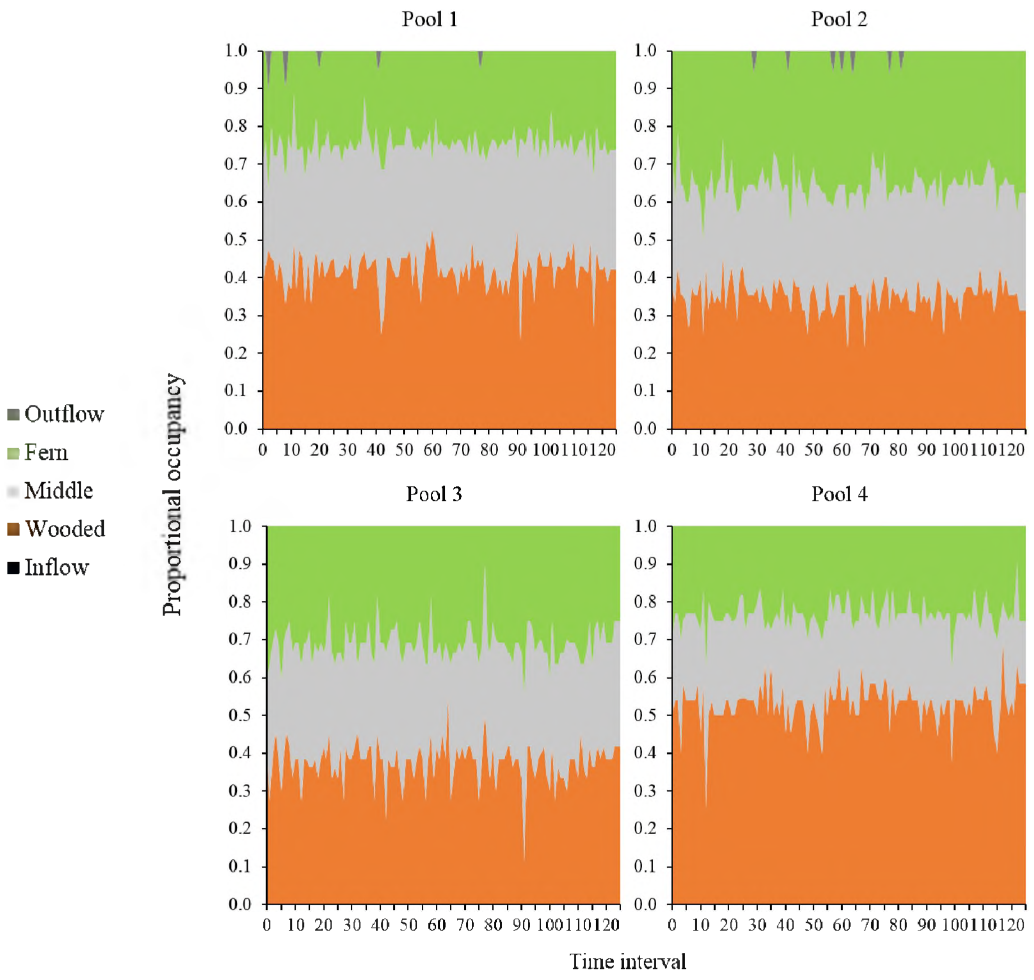

Figure 3.8. Proportion of *Sandelia capensis* found in each habitat (inflow, fern, middle, woody and outflow) across four pools (1, 2, 3 & 4) in a headwater tributary of the Swartkops River, Eastern Cape, South Africa.

Due to the large number of points in the dataset, it was decided to continue with the ANOVA analysis on the raw proportional occupancy data. The analysis (pool and habitat) showed that the habitat (*two-way ANOVA*; F (4, 12) = 10445.1, $p < 0.001$) and the interaction term between habitat and pool were significantly different (*two-way ANOVA*; F (12, 2460) = 201.9, $p < 0.001$). There was no significant difference in proportional occupancy between pools (*two-way ANOVA*; F (3, 2460) = 0.00, $p = 1$). Proportional occupancies of *S. capensis* across habitats was found to be significantly different (*ANOVA;* F (4, 2475) = 5294, $p < 0.001$).

Proportional occupancies for *S. capensis* were grouped for each habitat over the four pools, it was found that there was a significant difference (*ANOVA*; F (4, 15) = 77.23, $p < 0.001$) in Σ proportions over the five habitats (Fig 3.9.). It is evident to see there is a large difference in proportional occupancy found across all five habitats with the woody debris having the highest proportion occupancy (± 0.45) and the inflow and outflow having comparatively low proportion occupancy (< 0.1, Fig. 3.9.). A post-hoc Tukey test on proportional occupancy per habitat showed that all habitat interactions, except the one between the inflow and outflow and the fern root and middle habitats, were significantly difffernt ($p < 0.01$).

3.3.4 Behaviour

Video footage from Pool 4 was analysed to evaluate the behaviour of *P. afer* and *S. capensis* in the different habitat types. Comparisons of the behaviours observed are shown in Table 3.2. and Table 3.3. For *P. afer*, there were only two instances where one of the four behaviours were not recorded. No evidence of refuge or schooling was found in either the inflow or outflow habitats. Foraging was confirmed in all five habitats where *P. afer* were observed 'picking' on the substrate, presumably to consume prey items. Resting was observed in all five habitats. For *S. capensis*, woody debris was utilised the most for refuge and resting, followed by fern root.

A Chi-Square test of independence was calculated to compare the frequency of behaviour observed in each habitat for *P. afer*. A significant difference was found for all behaviours (*Chi-Square*, x^2 (12) = 69.77, $p < 0.001$) between habitats. For *S. capensis*, a Fisher's exact test comparing foraging and refuge in the woody and fern habitats and resting and schooling in the middle and outflow, showed there was no significant difference in behavioural observations ($p > 0.05$).

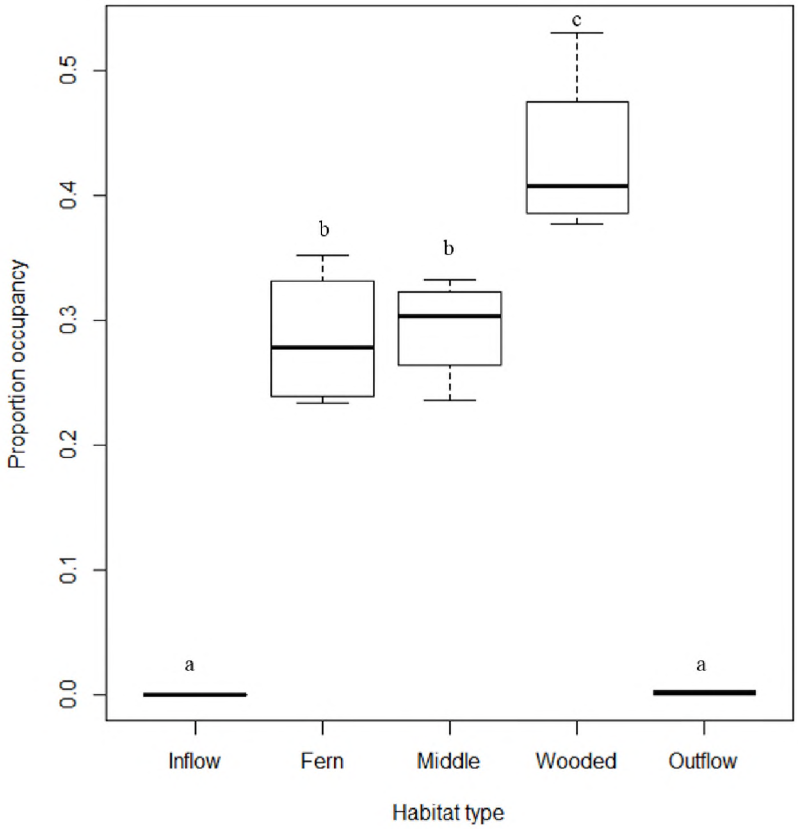

Figure 3.9. Summed proportional occupancy of *Sandelia capensis* in five habitat types within four pools of the Fernkloof headwater tributary of the Swartkops River, Eastern Cape, South Africa (lower case letters (a-c) represent where significant differences (tested with an ANOVA) were found).

Table 3.2. Frequency of behaviours counted for *P. afer* across five habitat types in the Fernkloof tributary in the Eastern Cape, South Africa

Habitat	Foraging	Refuge	Resting	Schooling	Total
Inflow	4	0	2	0	6
Woody	15	16	10	4	45
Middle	2	1	18	21	42
Fern	13	19	8	1	41
Outflow	2	0	1	0	3

Table 3.3. Frequency of behaviours exhibited by *S. capensis* across five habitat types in the Fernkloof tributary in the Eastern Cape, South Africa

Habitat	Foraging	Refuge	Resting	Schooling	Total
Inflow	0	0	0	0	0
Woody	3	7	4	0	14
Middle	0	0	2	1	3
Fern	1	4	3	0	8
Outflow	2	0	1	0	3

3.4 Discussion

From this study, it was evident that the use of videos and still photographs were both viable options when sampling headwater streams with action cameras. The two species sampled here were found to prefer similar habitat types with both species utilising different habitats for different reasons. *Pseudobarbus afer* were found to prefer the middle habitat in each pool followed closely by woody debris where they were observed mainly foraging and making use of the woody debris for refuge. *Sandelia capensis* were found to favour the woody debris over the other habitats. They were found to be using the woody debris mainly for refuge.

A strong correlation between MaxN estimates derived from still photographs and videos was found as well as MaxN estimates derived from the two methods did not differ significantly. This result is an indication that still photographs could be used in place of videos for sampling abundance in headwater streams. Not only will this drastically reduce analysis time, but it also increases the sampling potential with an action camera. When an action camera takes a photograph, it uses less battery than when constantly filming, therefore, the action camera can be utilised for an extended time when set to take photographs. Furthermore, less storage space is required for still photographs than that required by videos.

The high proportional occupancies for *P. afer* in woody debris and fern root areas may be attributed to their foraging tactics as they were often found feeding in these habitats. However, these habitats can also provide *P. afer* with structure for refuge. *Pseudobarbus afer* feed on small macroinvertebrates, algae and detritus material (Skelton, 2001) which are in abundance in the woody debris and the fern roots. This selective foraging behaviour may be a learned behaviour based on their needs (Odling-Smee *et al.*, 2011) for example, *P. afer* have learned that food is available in these respective habitats, so they return to them to forage. Food availability at the inflow and outflow may be reduced in comparison to the other areas of the pools. The shallow nature of the inflow and outflow habitats, reduce algal growth and the accumulation of detritus materials thereby possibly reducing the value of these habitats for foraging. In this case, the woody debris and fern root areas may be critical habitats as without these areas there may be limited food sources for *P. afer*.

Under laboratory conditions, *P. afer* tended to prefer grass and pipe habitat types with open water habitat types being the least favoured (Kadye & Booth, 2014). However, the data collected *in situ* for this chapter showed that *P. afer* favoured the deep middle habitat over the other four habitat types. The artificial setting of Kadye & Booth's experiments may have resulted in *P. afer* hiding rather than exhibiting the behaviours observed in their natural setting. The typical schooling behaviour that was observed in *P. afer,* especially in the deep middle section, could be a predator-avoidance strategy. Some fish are well equipped with sensory structures (such as their lateral-line system and good eyesight) that allow them to rapidly transfer information to one another (Brown & Laland, 2011). Members within a school can rapidly transfer information within the school about an approaching predator, while information transfer to individuals outside the school is less efficient.

Similarly, high proportional occupancies of *S. capensis* found in the woody debris and fern root habitats could be attributed to being a species that prefers cover over open water (Ellender *et al.* 2011), therefore seeking refuge or cover within structure. A low proportional occupancy of *S. capensis* found in the outflow, and no *S. capensis* found in the inflow, could be attributed to a preference for slower-flowing water (Skelton, 2001). Habitats, such as woody debris and fern root, may also be favoured by *S. capensis* due to the abundance of resources that they may offer such as smaller fishes (hiding in these areas for refuge; possibly juvenile *P. afer*) and other small invertebrates or insects. Furthermore, *S. capensis* is an ambush predator and the refuge offered by these more complex habitats could aid in their predatory success.

Pseudobarbus afer and *S. capensis* were found coexisting in three of the five habitats in each of the pools (woody debris, fern root and deep middle). This is evidence that the two-species forage in the same habitats, but further analyses are required to specifically determine the degree of trophic niche overlap between the species through comparisons of gut content analyses and the use of stable isotope analysis. Although they were found in the same habitats, *P. afer* and *S. capensis* were found to favour different habitats.

Although the main predator in the headwater streams of the study area is the African longfin eel *Anguilla mossambica* (Peters, 1852), there may be other threats to *P. afer* and *S. capensis,* such as birds or otters (Kadye & Booth, 2014). Therefore, the choice for *P. afer* to school for most of the time observed, suggests that they are practicing predator avoidance (Magurran, 1990). Due to *S. capensis* being a cryptic and structure-orientated species favouring habitats that provide refuge, they are at a lower risk of being targeted by terrestrial predators (Swartz & Impson, 2007). Terrestrial predators, such as diving or wading birds, may target fish in shallower waters (Kadye & Booth, 2014) such as the inflow or outflow. This may explain why *P. afer* also appeared to avoid the inflow and outflow of pools. This predator avoidance strategy may also be attributed to their genetic predisposition (Gornman, 1988) and not necessarily because of exposure to predators. Alternatively, the presence of other fishes in these pools may affect the behaviour of *P. afer* and their choice of habitat utilisation. The Cape kurper *Sandelia capensis*, rapidly colonise certain habitats within these pools and may outcompete *P. afer* in certain habitats, therefore, reducing the utilisation by *P. afer*.

Pseudobarbus afer and *S. capensis* exhibited boldness numerous times in this study. Every time an action camera was placed into the water, fish would inspect it. This is classified as inspection of a novel object (Budaev & Brown, 2011) and relates to the bold end of the shy-bold continuum (Wilson *et al.*, 1994). A second boldness characteristic exhibited by *P. afer* was the time spent in an open habitat. *Pseudobarbus afer* were most abundant in the middle habitat, which can be classified as an open habitat, whereas *S. capensis* were recorded relatively few times in open habitats. *Sandelia capensis* were, however, prone to exhibiting inspection of a novel object and were found to inspect the camera throughout the whole study.

Human-mediated impacts (flow modification and habitat destruction/degradation) are degrading many freshwater systems and, therefore, habitats within these systems. It is crucial that scientists explore these habitats and how fish species are using them to gain a better understanding of alternative methods to conserve both habitats and fish fauna found within these waterbodies. This study is one of the first studies in South Africa to make use of action cameras to document habitat utilisation by these specific species and has provided insight into the habitat preference of *P. afer* and *S. capensis*. The information gathered on both species discussed in this chapter is of interest to researchers and the study site provides an opportunity to monitor two imperilled freshwater fish species in a near pristine environment and make deductions about both fish behaviour and habitat utilisation.

CHAPTER 4

ARTIFICIAL HABITAT UTILISATION BY *PSEUBARBUS AFER* AND *SANDELIA CAPENSIS*

4.1 Introduction

The wide disparity of habitats within freshwater systems provide different services to fish species. Freshwater fishes exhibit a high variation in morphologies, suggesting that they have evolved to exploit specific habitats (Wikramanayake, 1990; Winemiller, 2008). Some habitats may be viewed as critical habitats (Rosenfield & Hatfield, 2006) and are crucial to the survival of all species. There is usually a positive relationship between critical habitats and the services provided by the habitat and the fish population size (Rosenfield & Hatfield, 2006). For example, habitat that provides refugia to a species can be viewed as a critical habitat. An area of refugia can be defined as an area where environmental factors temporally or spatially reduce the negative effects of disturbances to the area (Magoulick & Kobza, 2003). Habitats that display these traits are ecologically important for the survival and wellbeing of organisms and they should, therefore, be prioritised for protection against human-driven alteration.

Globally, freshwater systems are facing a variety of human-mediated threats and highly important endemic species are at risk of population decline and potential extinction (Dudgeon *et al.*, 2006). Some of the stressors are significant contributors towards the destruction of freshwater systems and include habitat alteration (through flow modification or over-abstraction of water) and the introduction of non-native invasive species (Saunders *et al.*, 2002; Dudgeon *et al.*, 2006). Ultimately the two stressors are linked and an increase in habitat modification or destruction can lead to an increase in biological invasions (Mack *et al.*, 2000; Dudgeon *et al.*, 2006; Matsuzaki *et al.*, 2013). It is likely that an increase in anthropogenic stressors and habitat alterations will result in declines in the productivity of these fresh waterbodies (Pratt, 1994). Therefore, focus should be placed on trying to alleviate these problems so that the rate of recovery for each freshwater system is higher than the rate of destruction, and this is particularly relevant for habitats and areas that are important to endemic species (such as the CFE). If the threat to the endemic species in question is not overfishing, the survival rate of these endemic species can be improved by either eradicating the non-native invasive species or by providing various resources, such as refugia (Schlaepfer *et al.*, 2005;

Yokomizo *et al.*, 2007; Westhoff *et al.*, 2013) to enhance the survival and success of the endemic species (Yokomizo *et al.*, 2007; Simberloff *et al.*, 2014).

With the increase in modifications to freshwater systems, threats to freshwater systems and fish stocks will, therefore, be impacted, particularly those found in headwater streams. Freshwater fishes make use of various habitat types throughout their life cycle (Schlosser, 1991) i.e. a favoured habitat for spawning or juveniles (such as nursery areas). Therefore, conservation strategies for species at risk need to protect specific habitats within waterbodies and the connectivity between these habitats. Furthermore, habitats within a certain waterbody may be utilised by more than one species and, in such cases, conservation strategies can be designed to protect a variety of species. If threats to habitats found within fresh waters are unavoidable, alternative solutions must be sought to alleviate the long-term impacts on fish populations. This can be achieved through the replication of the natural habitat using artificial materials. This approach may aid in the practical conservation management of a fish species and the use of artificial habitats has been increasingly investigated for this purpose (Nash *et al.*, 1999; Knaepkens *et al.*, 2004).

Globally, artificial structures have been used to influence the behaviour and ecology of a variety of aquatic organisms and have ranged from small-scale modifications to large structures that are deployed over areas of seafloor or freshwater substrate (Seaman & Sprague, 1991). The original rationale for introducing these structures has been to improve environmental quality or provide structure. For the introduction of artificial habitats to be successful, a process of decision making that includes defining the users and purposes (Seaman & Sprague, 1991; Seaman, 1996) of the artificial habitat and post-deployment evaluations (Wilding & Sayer, 2002) of the habitat use needs to be followed.

Furthermore, the success rate of these artificial structures is dependent on the colonisation rate by the fish species in question (Pratt, 1994). It is crucial to monitor how a species reacts to the introduction of an artificial habitat and important factors that have been identified include habitat size and its distance from the source populations (Pratt, 1994). The use of artificial habitats within freshwater systems may be limited by the size of the waterbody in question, i.e. a large reservoir can hold more artificial habitat than a pool within a headwater stream and, therefore, the rate of success needs to be measured on a relative scale. In contrast, different observations on species utilisation of an artificial habitat can be made by conducting experiments in a laboratory scenario versus a scenario *in situ*.

Santos *et al.*, (2008) evaluated the use of artificial habitat structures as a tool for fish rehabilitation in a neotropical reservoir. Artificial structures were created at varying degrees of complexity (dense, middle and control) with polyvinylchloride (PVC) piping and polypropylene ribbons being placed at two different positions within the water column (midwater and bottom). The chosen area for placement of artificial habitats was within a protected area (to prevent interference from non-authorized individuals), in deep water that had high transparency and had low (or depleted) amounts of other submerged macrophytes. Observations of fish colonisation on each artificial structure were carried out underwater by two divers. Preliminary observations showed that a period of 15 days was sufficient for periphyton and fish colonisation to occur. It was found that, of the nine fish species recorded utilising the artificial habitat, the majority were juveniles or forage fishes. It was also found that the percentage frequency of occurrence was higher at structures that had more complexity compared with those with low levels of complexity (Santos *et al.*, 2008).

In a laboratory-based experiment, Kadye and Booth (2014) made use of artificial habitats to determine the diel patterns and favourable habitat for the Eastern Cape redfin minnow *Pseudobarbus afer* (Peters, 1864). *Pseudobarbus afer* were collected in the field and, following an acclimation period, were placed into tanks containing half-cylinder PVC pipes (that represented refugia) and polyethylene strips (that represented aquatic grass). It was found that *P. afer* made use of the artificial habitat structures more than they utilised the 'open' habitat in the middle of the tank where no habitat was present. Although this experiment was not intended to provide conservation management outcomes, it showed that the natural habitats occupied by *P. afer* could potentially be successfully replicated using artificial materials.

In this chapter, the habitat use and behavioural responses of *P. afer* and the co-occurring Cape kurper *Sandelia capensis* (Cuvier, 1829) were evaluated following the introduction of an artificial habitat into the four pools in the Fernkloof tributary as discussed in the previous chapter. As shown in Chapter 3, *P. afer* and *S. capensis* have varying habitat preferences, however, these can overlap at times and the two species can be seen coexisting in similar habitats (also shown in Ellender *et al.* (2012) and Kadye and Booth (2014) where both species were detected in the same habitats). Both species are conservation priorities due to the various threats (pollution, habitat degradation and flow modification) that they face. The present chapter sought to determine whether it was possible to monitor both species' habitat preference *in situ* following the introduction of artificial habitat using action cameras. Artificial habitats could be used as a step towards conserving both species. Following on from Chapter 3 and work of Kadye and Booth (2014), the current chapter assessed: (1) the difference in habitat preference between *P. afer* and *S. capensis* following the introduction of an artificial structure; and, (2) whether the introduced artificial structure was favoured by either, or both, of the species over the naturally occurring habitats. This was achieved using action cameras *in situ* assessing habitat utilisation and making inferences on both species' behaviour in each habitat.

As seen in results presented in Chapter 3, it was hypothesised that *P. afer* would favour the middle habitat over the other four habitats and that *S. capensis* would favour habitats that provided a certain level of refuge. It was also hypothesised that both species would utilise the artificial habitat placed into each of the pools and that both species would be seen going in and out of the pipes.

4.2 Methods and materials

4.2.1 Study area

This study took place in the same four pools used in Chapter 3 (Fig. 3.2.) on the Fernkloof tributary (33°43' S, 25°17' E) of the Swartkops River. Data collection took place over four months between February and May 2017. In February 2017, artificial habitats were placed within each of the four pools used in the study discussed in Chapter 3.

After a one-month acclimation period, four separate data collection trips took place approximately three weeks apart. These four pools were selected as they had been used previously for an analysis of habitat utilisation and behaviour (see Chapter 3), contained all five habitat types of interest, and were suitable for underwater photographs. Water physico-chemical parameters were taken in the same fashion as in Chapter 3 (Table 4.1) with the pool dimensions being presented in Table 3.1.

4.2.2 Artificial habitat

Artificial habitats were created from nine 30 cm long PVC pipes with a diameter of 6 cm and connected with cable ties (Fig. 4.1). Chain was fed through the bottom pipes of each habitat to weigh artificial habitat down (as indicated by arrows in Fig. 4.1). The chains were attached to 0.5 m lengths of 5 mm diameter threaded rods, which were driven into the substrate, to ensure the artificial habitats would remain in position in the event of strong flows. One artificial habitat was placed into each of the four pools so as not to interfere with the other habitats.

Table 4.1. Summary statistics produced from physico-chemical readings from the four visits shown as the mean reading (a total of three readings for each parameter) per pool ± SD per visit. GPS locations and pools sizes can be seen in Table 3.1.

Visit	Pool	Water temperature (°C)	Turbidity (NTU)	pH	Conductivity (µs/cm)
1	1	19.7 ± 0.2	0.68 ± 0.29	5.31 ± 0.03	229 ± 2
1	2	19.7 ± 0.1	0.33 ± 0.05	5.17 ± 0.04	230 ± 2
1	3	19.3 ± 0.1	0.33 ± 0.04	4.77 ± 0.18	232 ± 2
1	4	20.8 ± 0.1	0.40 ± 0.03	5.41 ± 0.51	239 ± 1
2	1	19.6 ± 0.2	0.49 ± 0.14	4.94 ± 0.06	231 ± 1
2	2	19.7 ± 0.1	0.21 ± 0.02	5.44 ± 0.09	238 ± 4
2	3	20.2 ± 0.1	0.24 ± 0.07	5.07 ± 0.05	242 ± 1
2	4	19.7 ± 0.1	0.25 ± 0.02	6.20 ± 0.04	247 ± 3
3	1	18.4 ± 0.2	0.58 ± 0.03	4.87 ± 0.24	237 ± 2
3	2	18.1 ± 0.1	0.49 ± 0.25	5.17 ± 0.04	231 ± 2
3	3	18.0 ± 0.2	0.35 ± 0.06	5.01 ± 0.14	215 ± 4
3	4	18.4 ± 0.1	0.46 ± 0.21	5.34 ± 0.17	221 ± 1
4	1	18.5 ± 0.1	0.49 ± 0.11	4.50 ± 0.17	234 ± 4
4	2	18.2 ± 0.3	0.36 ± 0.07	5.55 ± 0.17	234 ± 5
4	3	18.4 ± 0.2	0.32 ± 0.03	5.06 ± 0.14	239 ± 2
4	4	18.2 ± 0.2	0.36 ± 0.07	5.61 ± 0.02	229 ± 7

4.2.3 Data collection

Filming took place on four different occasions. Six GoPro Hero 3+ action cameras (GoPro Inc., United States of America) in waterproof housings were mounted on gorilla tripods and set at the six preselected habitats in each pool approximately 1.5 m away from the habitat. The flow of the water was low at the time of this study and therefore, tripods did not need extra weighing down and were able to just be placed in the substrate. Following recommendations from Chapter 2 which showed that 15 minutes was adequate filming time, the cameras were set to film for 25 minutes at a frame rate of 720 fps and 12-megapixel resolution, concurrently taking photographs every five seconds. The extended filming time provided more counts for habitat use and was chosen based on the minimum battery life of the cameras.

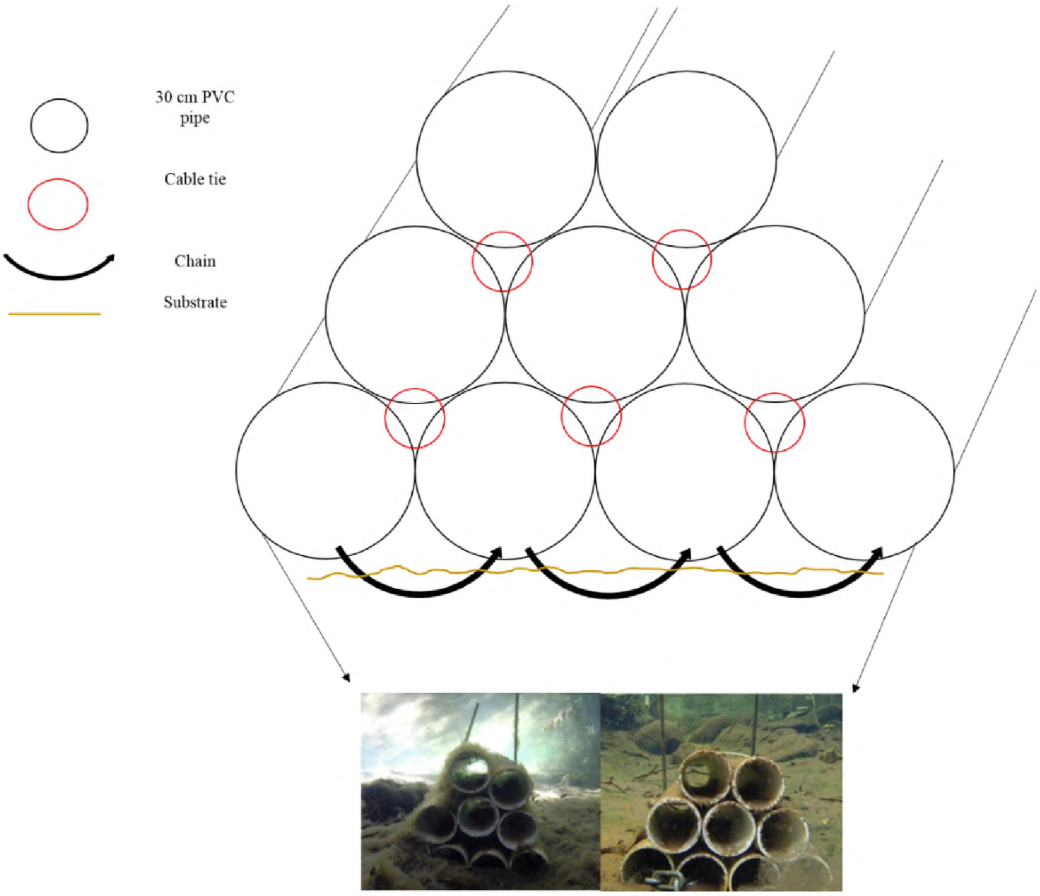

Figure 4.1. Schematic diagram and photographs of artificial habitat that was created out of 30 cm (length) PVC piping (large circles) attached together with cable ties (small circles) and weighed down with chains in the bottom row (arrows). Each habitat was replicated four times and placed in four separate pools within the Fernkloof tributary of the Swartkops River, Eastern Cape, South Africa.

4.2.4 Habitat utilisation

Initial acclimation period of three minutes was discarded for each camera, thereafter photographs were viewed in Windows Photo Viewer (Microsoft Inc.). Still photographs were analysed in the same fashion as described in Chapters 2 and 3, using the MaxN metric (see 2.2.5 & 3.2.5). Once again, proportional occupancy was used in preference to the raw MaxN values to account for different numbers of fish in each pool. To calculate a proportion occupancy, the mMaxN values for each habitat per time interval were summed across habitats (ΣMaxN). Proportional occupancy for each habitat for each time interval was calculated as the habitat mMaxN divided by the ΣMaxN for the corresponding time interval. Readings were averaged over the four sampling periods to give an average proportional occupancy per pool.

Proportional occupancy of each habitat by *P. afer* and *S. capensis* in each pool were checked for normality using the Shapiro-Wilk test. The data were found to be significantly different from normality. An acrsine-square root transformation was applied but the transformed data were still significantly different from normality. Due to the large number of records, it was decided to use an ANOVA for the analyses even though the data did not meet the normality criterion (Liu, 2015). A two-way ANOVA was used to test whether there were significant differences in proportional occupancy between pools and habitat type for both species. As there was no significant relationship for pools, but a significant result for the habitat type and the pool-habitat type interaction, a one-way ANOVA was used to test that there were significant differences in *P. afer* and *S. capensis* proportional occupancy across habitats. Subsequently, a two-way ANOVA was used to test whether there was a significant difference in proportional occupancy for each species between habitat types over the four visitations.

4.2.5 Multivariate analysis of mMaxN estimates and water quality parameters

Following these analyses, the relationship between fish density (mMaxN) within habitats was explored further for both *P. afer* and *S. capensis*. For these analyses, the inflow and outflow habitats were excluded due to the low occupancy of both species in these habitats. Additional variables, including water depth, pH, conductivity and water temperature were included as predictors but all parameters, except for water temperature and water depth, were found to be constant over the four visits and were excluded from the analysis. To control for any unknown effect unique to a pool or visit, visitation date was nested within pool as random effects. In addition, the density (mMaxN) of the other species was included to evaluate whether its presence influenced the density of the species being evaluated.

Depth was found to be associated with habitat type. An ANOVA based on a linear mixed effects model was used to determine whether depth influenced habitat type, a linear mixed effects model with random effects 'visit' nested within 'pool' was constructed using the lmer function in the R package lme4 (Bates *et al.*, 2015) and an ANOVA was conducted on the linear mixed effects model using the ANOVA function in the R package car (Fox & Weisberg, 2011). The test was conducted using a type II Wald F tests with Kenward-Roger df. A Tukey test was conducted *post hoc* on the best model using the glht function from R package multcomp (Horthorn *et al.*, 2008).

Upon finding that water depth had a significant effect on habitat, three further models were constructed using a linear mixed effects model to determine the best model describing the mMaxN estimates for the two-species found in different habitats. These models were the “All” model (predictors: habitat, temperature, depth and other species), “Without Depth” model (predictors: habitat, temperature and other species) and the “Without Habitat” model (predictors: temperature, depth and other species). The statistical analyses were as described above for depth. The model with the lowest Akaike Information Criterion (AIC) was chosen as the best model.

4.2.6 Behaviour

All videos were viewed in Windows Media Player (Microsoft Inc.) as described in Chapters 2 and 3 (see 2.2.5 & 3.2.5). The first three minutes of each video was discarded as the acclimation period. Analysis of videos was done separately per habitat and per pool for each of the four visitations.

Fish behaviours were given a score of one every time they were observed. These included foraging, refuge, schooling or resting. Behaviour counts were then grouped per habitat for each pool (resulting in one score for a specific habitat in each pool for the four readings). *Pseudobarbus afer* and *S. capensis* behaviour were analysed across habitats types using a Chi-Square test of independence to test whether there were any significant differences in behaviours observed.

All data analyses were conducted using R 3.4.1 statistical software (R Development Core Team. 2016) through RStudio 0.99.902 (RStudio, Inc.) and MS Excel 2013 (Microsoft). All statistical analyses used $p = 0.05$ to determine statistical significance.

4.3 Results

Pools were characterised as areas that were deep along the river with substratum that was visibly different to that in the inflow or outflow section. The inflow and outflow to each pool mainly contained boulders (> 256 mm) while substratum in each pool ranged from gravel (10 – 64 mm) to cobble stones (64 – 256 mm) with pockets detritus and decaying plant material. Flow between pools was low throughout the whole study.

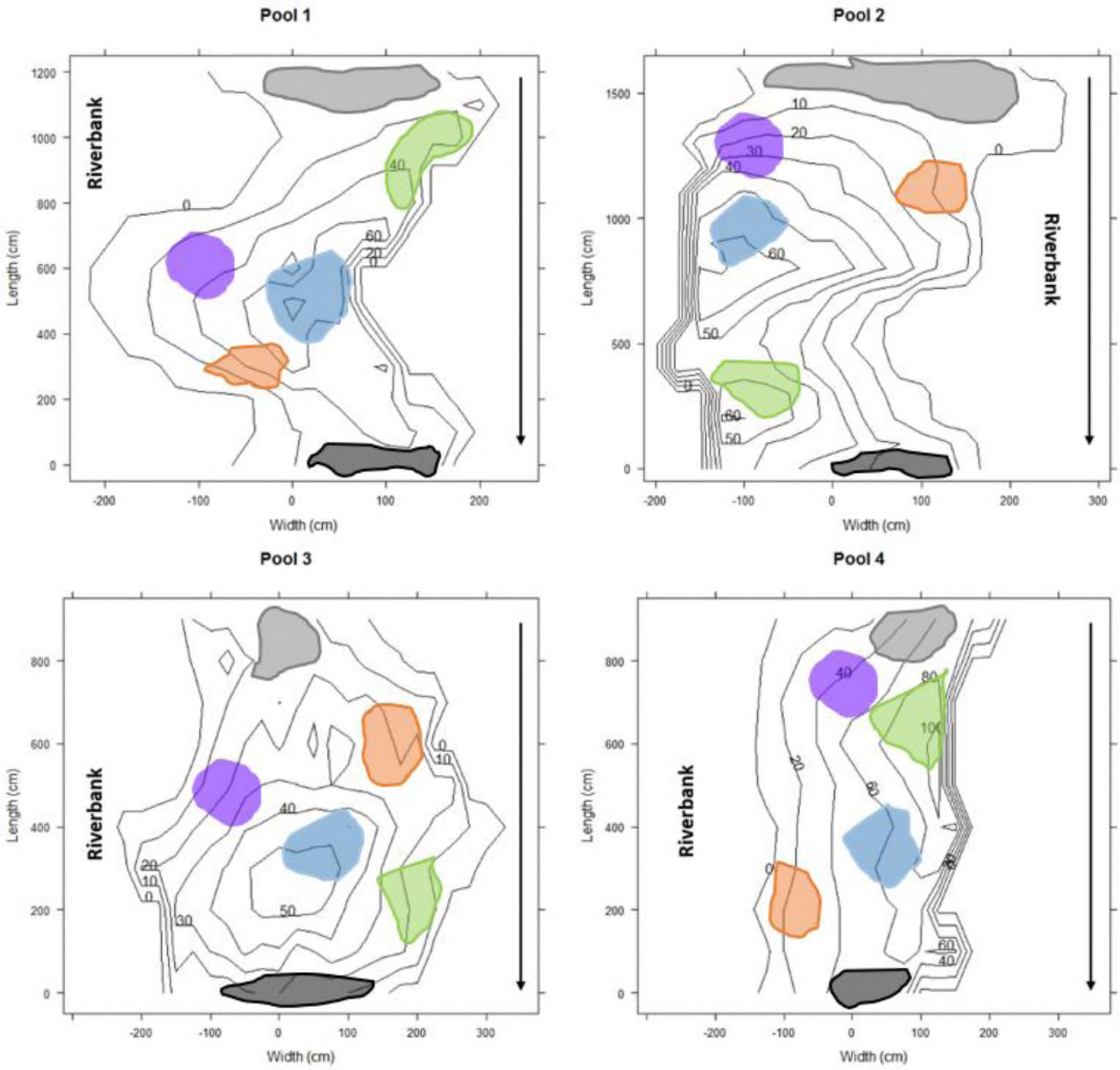

Figure 4.2. Contourplots (like those shown in Figure 3.4) of the four pools used in this study. Depths are indicated by contour lines and different habitat types and sizes are indicated by coloured shapes (grey = inflow, orange = woody, green fern root, blue = middle, purple = artificial and black = outflow).

4.3.1 Habitat preference

The middle habitat was favoured by *P. afer* followed by the woody debris and then the fern root areas (Fig. 4.3). Relatively few *P. afer* were detected in the outflow and inflow sections of the pools. *Pseudobarbus afer* counts in artificial habitats were also low. There was a significant difference for habitat type (*two-way ANOVA*; F (5, 84) = 110.71, $p < 0.001$) and the interaction term between habitat type and pool (*two-way ANOVA*; F (15, 1008) = 31.21, $p < 0.001$), but not for pool (*two-way ANOVA*; F (1, 84) = 0, $p = 1$). Due to the large sample size, pool was discounted as a factor. Analyses across habitat types showed a significant difference in proportional occupancy (*ANOVA*; F (5, 1026) = 3997, $p < 0.001$). When proportional occupancies by habitat type were separated by visit (Fig 4.4), no significant difference was found within the respective habitat types over the different visits (*two-way ANOVA*; F (15, 72) = 1.361, $p = 0.191$).

There were no significant differences found in proportional occupancies within habitats over the different visitations (all $p > 0.05$). During visitation 4 (D), *P. afer* proportions were low in the inflow, fern, artificial and outflow habitat while they were high in the woody and middle habitats. This indicates possible movement between habitats. A decrease in proportion occupancy from visit 1 to 4 (A-D) in all habitats other than the woody and artificial habitat, indicates that *P. afer* started frequenting other habitats more regularly over the study other than the favoured middle.

Sandelia capensis favoured the woody debris habitat in preference to the five other habitats (Fig. 4.5). This was followed by the middle, fern root and artificial habitats. There were no *S. capensis* recorded in either the inflow or the outflow areas. There was a significant difference for habitat type and the interaction term between habitat type and pool (*two-way ANOVA*; F (15, 1008) = 79.11, $p < 0.001$), but not for pool ($p = 1$). Due to the large sample size, pool was discounted as a factor. There was a significant difference in proportional occupancies across habitats (*ANOVA*; F (5, 1026) = 3926, $p < 0.001$). When proportional occupancies were separated by visit and habitat (Fig 4.6), no significant difference in proportional occupancies within the respective habitat types over the different visits (*two-way ANOVA*; F (15, 72) = 1.256, $p = 0.253$).

During visitation 1 (A), *S. capensis* proportions are low in the inflow, woody, middle, fern and outflow while they are high in the artificial habitat. This indicates that there is possible movement between habitats. An increase in the proportion occupancy from visit 1-4 (A-D) in the woody habitat, indicates that *S. capensis* started using the woody habitat more frequently during this study. There were no significant differences found in proportional occupancies within habitats over the different visitations (all $p > 0.05$).

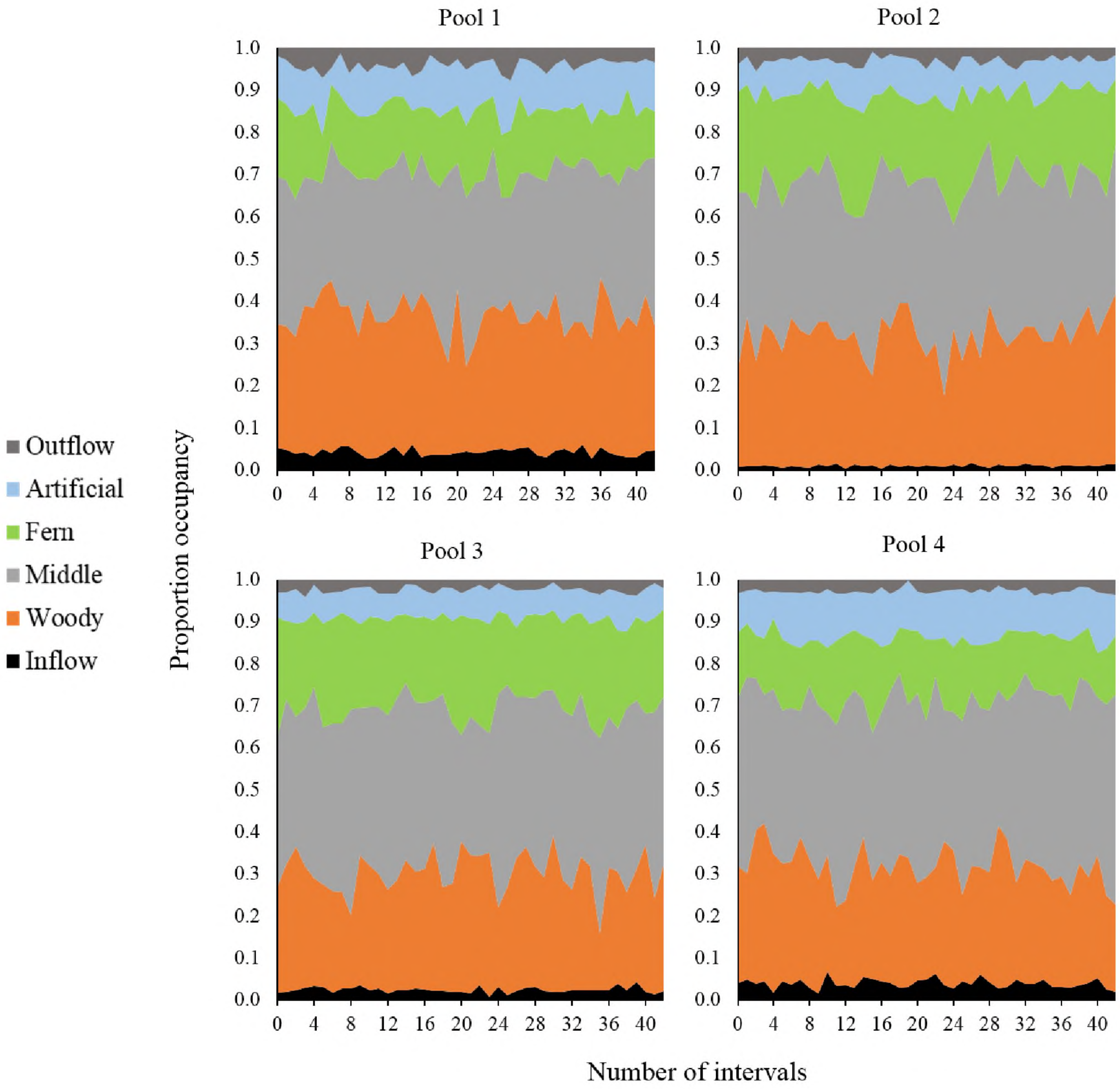

Figure 4.3. Proportion occupancy of *Pseudobarbus afer* across six habitats (outflow, artificial, fern root, middle, woody debris and inflow) in four pools (1 - 4) of the Fernkloof tributary of the Swartkops River in the Eastern Cape, South Africa. Each time interval, is equivalent to 6 photographs or 30 seconds.

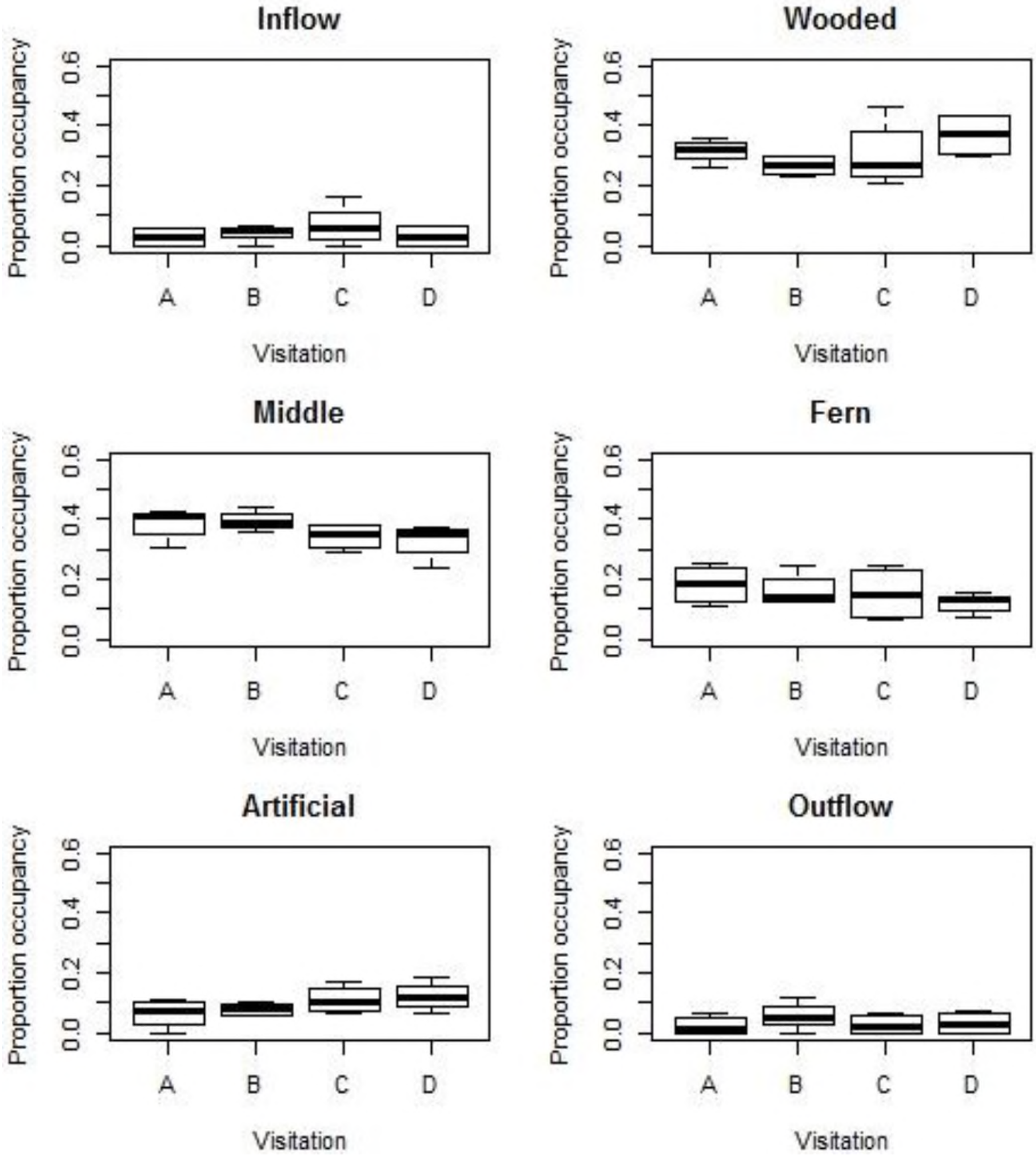

Figure 4.4. Proportional occupancy of *Pseudobarbus afer* in six habitat types within four pools of the Fernkloof headwater tributary of the Swartkops River, Eastern Cape, South Africa. A, B, C and D represent the four different visitations. The four different visitations took place between February and May 2017.

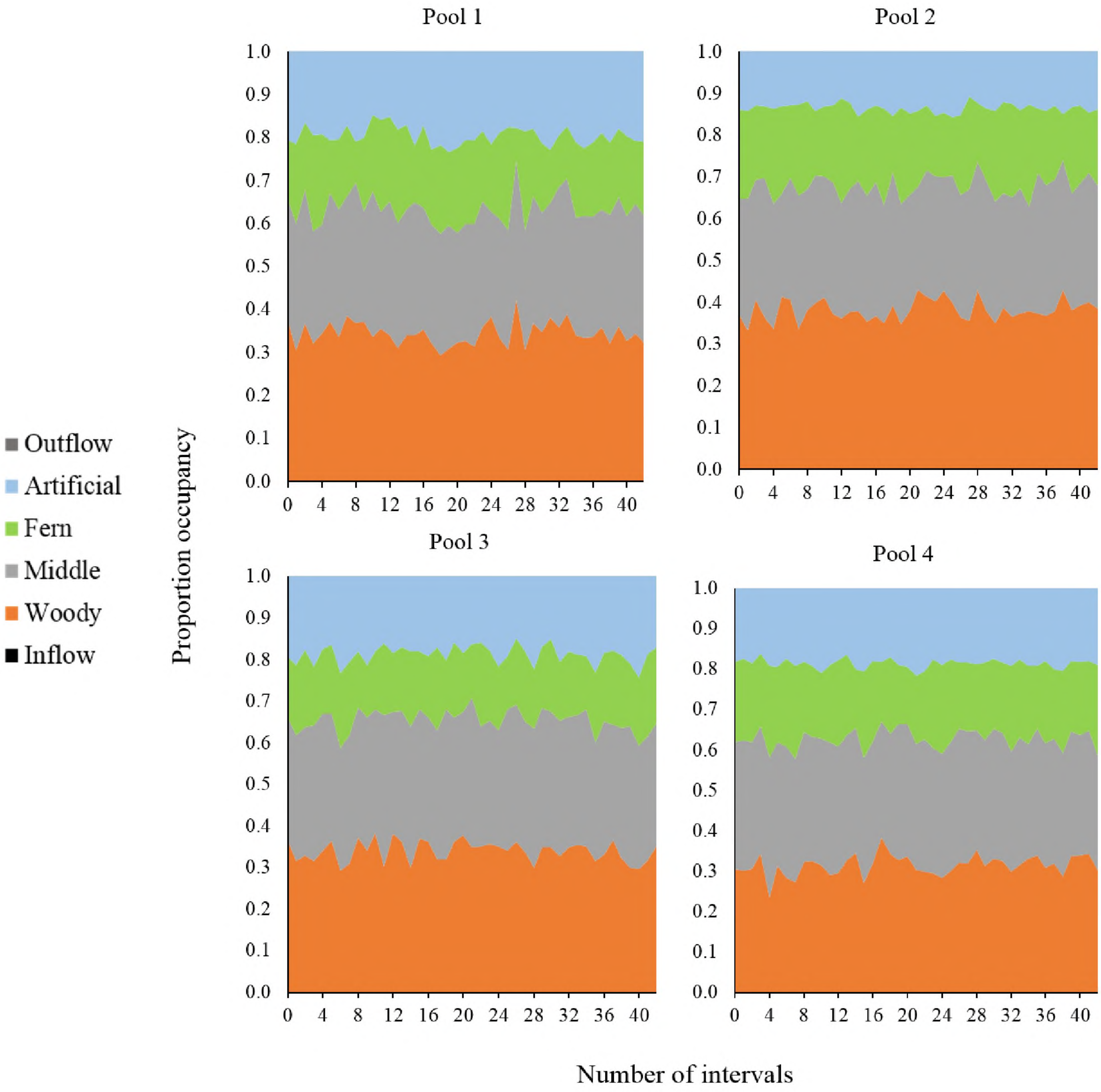

Figure 4.5. Proportional occupancy of *Sandelia capensis* across six habitats (outflow, artificial, fern root, middle, woody debris and inflow) in four pools (1 - 4) of the Fernkloof tributary of the Swartkops River in the Eastern Cape, South Africa. Each time interval, is equivalent to 6 photographs or 30 seconds.

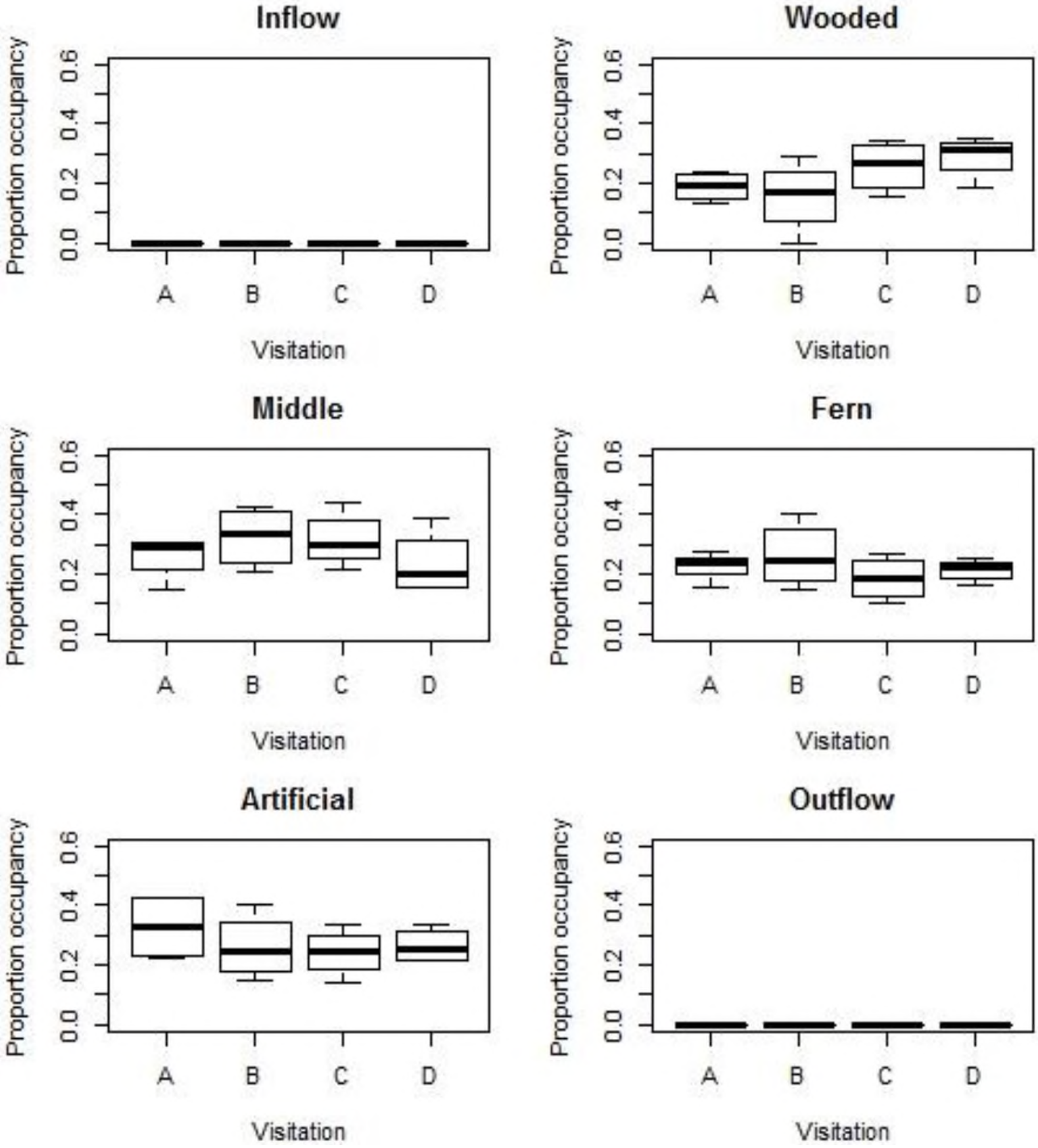

Figure 4.6. Proportional occupancy of *Sandelia capensis* in six habitat types within four pools of the Fernkloof headwater tributary of the Swartkops River, Eastern Cape, South Africa. A, B, C and D represent the four different visitations. The four different visitations took place between February and May 2017.

4.3.2 Multivariate analysis

In the preliminary analysis of the predictors used for the multivariate analysis, there was a relationship between water depth and habitat type. The middle habitat was significantly deeper than the other habitats (Fig. 4.7). The linear mixed effect model constructed to evaluate this relationship showed that depth had a significant effect on habitat type (*ANOVA;* F (3, 53) = 14.18, $p < 0.001$). It was, therefore, decided to evaluate three models to evaluate the interaction between depth and habitat further.

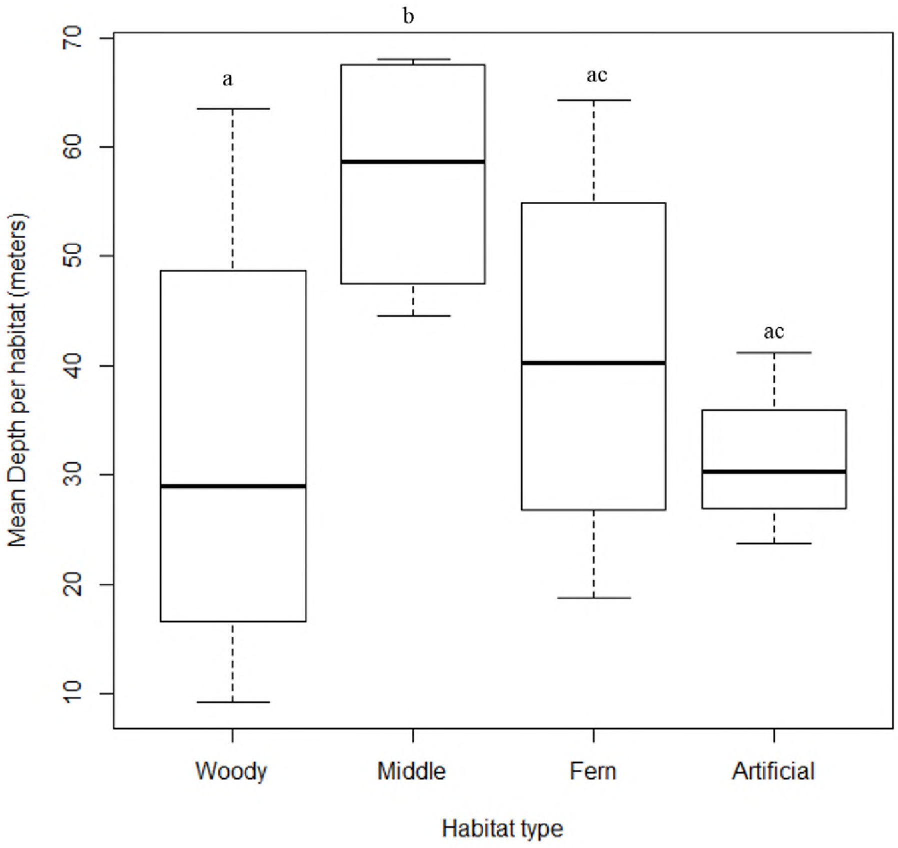

Figure 4.7. Mean depth (m) measured in four different habitat types in four pools in the Fernkloof tributary in the Eastern Cape, South Africa. Statistical significances between habitat types are indicated by lower case letters. Box and whisker plots with the same lower-case letter have no significant different to each other while box and whisker plots with different letters have significant difference to each other.

The multivariate analysis (Table 4.2) demonstrated that the model excluding depth, based on the lowest AIC value, was deemed the best model for *P. afer*. When all parameters were included it was found that habitat, water temperature and *S. capensis* parameters were all significant. When habitat was excluded it was found that water temperature and water depth were significant and when water depth was excluded it was found that water temperature, habitat and *S. capensis* were significant. From this it is evident that water depth is an important component of habitat but does not describe habitat in totality.

Like *P. afer*, the model for excluding water depth, based on the lowest AIC value, was deemed the best model for *S. capensis* (Table 4.3). When comparing the models, only the *P. afer* parameter was significant for the All and Without Depth models. None of the other parameters included were significant in any of the models. From this it was evident that the factors describing the density of *S. capensis* were not fully captured in the parameters evaluated and that habitat was not as strong an association for *S. capensis* as it was for *P. afer*.

Table 4.2. Results from an ANOVA using linear mixed effects models for *Pseudobarbus afer* mMaxN found in different habitats.

Model	**Habitat**	**Temperature**	**Depth**	***S. capensis***	**AIC**
All	$F_{3,52.19}=28.198$, **$p<0.0001$***	$F_{1,9.17}=6.993$, **$p=0.026$***	$F_{1,28.47}=0.012$, $p=0.913$	$F_{1,50.68}=4.916$, **$p=0.031$***	210.5
Without Habitat		$F_{1,5.01}=7.131$, **$p=0.044$***	$F_{1,40.17}=4.653$, **$p=0.037$***	$F_{1,38.81}=1.927$, $p=0.173$	258.0
Without Depth	$F_{3,51.7}=35.014$, **$p<0.0001$***	$F_{1,9.41}=6.969$, **$p=0.026$***		$F_{1,51.67}=5.050$, **$p=0.030$***	200.8

Table 4.3. Results from an ANOVA using linear mixed effects models for *Sandelia capensis* mMaxN found in different habitats.

Model	**Habitat**	**Temperature**	**Depth**	***P. afer***	**AIC**
All	$F_{3,52.50}=2.474$, $p=0.072$	$F_{1,35.62}=3.832$, $p=0.058$	$F_{1,26.03}=0.038$, $p=0.847$	$F_{1,55.79}=5.318$, **$p=0.025$***	90.9
Without Habitat		$F_{1,32.36}=2.806$, $p=0.104$	$F_{1,40.67}=0.007$, $p=0.936$	$F_{1,56.88}=3.100$, $p=0.084$	85.5
Without Depth	$F_{3,52.59}=2.507$, $p=0.069$	$F_{1,36.881}=4.004$, $p=0.053$		$F_{1,56.73}=5.435$, **$p=0.023$***	79.1

4.3.3 Behaviour

Behavioural counts revealed that *P. afer* utilised the fern habitat the most (Table 4.4.), followed by the woody debris, middle and then artificial habitat. *Pseudobarbus afer* were viewed foraging in fern and woody habitats feeding off detritus material that had settled on the fern roots and in the woody habitats. In the middle habitat, *P. afer* were mainly viewed schooling or resting. The inflow and outflow were utilised the least by *P. afer* as shown by the low counts. The artificial habitat was not used by *P. afer* for refuge, however, they were observed feeding off detritus material which had settled on the structures. A Chi-Square test of independence based on the behavioural counts showed that there was a significant difference in behaviour observed from the six habitats for *P. afer* (*Chi-Square*, x^2 (15) = 206.78, $p < 0.001$).

Table 4.4. Grouped behavioural counts over four visits of behaviour observed for *Pseudobarbus afer* across six habitats in four pools of the Fernkloof tributary of the Swartkops River in the Eastern Cape, South Africa.

	Foraging	Refuge	Resting	Schooling	Total
Inflow	10	0	5	0	15
Woody	41	33	21	13	108
Middle	6	3	35	54	98
Fern	57	62	19	7	145
Artificial	26	0	4	8	38
Outflow	7	0	5	0	12

Behavioural counts revealed that *S. capensis* utilised the artificial habitat the most (Table 4.5) followed by the woody debris, fern root and then middle. *Sandelia capensis* were viewed using the artificial habitat for refuge often entering a pipe and staying there for an extended period then exiting the pipe and entering a new pipe. *Sandelia capensis* used woody debris for foraging and refuge. The inflow and outflow habitats were not utilised by *S. capensis*. The artificial habitat was used most for refuge whilst woody debris was used most for foraging. A Chi-Square test of independence based on the behavioural counts showed that there was a significant difference in behaviour observed from the six habitats for *P. afer* (*Chi-Square*, x^2 (15) = 42.08, $p < 0.001$) and that behaviour counts were dependant on habitat.

Table 4.5. Grouped behavioural counts over four visits of behaviour observed for *Sandelia capensis* across six habitats in four pools of the Fernkloof tributary of the Swartkops River in the Eastern Cape, South Africa.

	Foraging	Refuge	Resting	Schooling	Total
Inflow	0	0	0	0	0
Woody	12	10	8	2	32
Middle	6	0	0	1	7
Fern	8	6	4	2	20
Artificial	4	42	4	8	58
Outflow	0	0	0	0	0

4.4 Discussion

From the study conducted in this chapter, it was found that *P. afer* and *S. capensis* utilised similar habitats in four pools in the Fernkloof tributary of the Swartkops River, South Africa. *Pseudobarbus afer* were found to favour the middle habitat while *S. capensis* favoured the artificial habitat. *Pseudobarbus afer* were observed using the artificial habitat for foraging and were observed picking at the detritus which had settled on the PVC pipes through the study. *Sandelia capensis* were observed more regularly using the artificial habitat for what appeared to be refuge. They were observed going in and out of the pipes numerous times and, often, more than one *S. capensis* utilised the artificial structures at the same time.

Pools within the Fernkloof tributary, exhibited a wide array of habitats. *Pseudobarbus afer* and *S. capensis* utilised different habitats for different purposes. *Sandelia capensis* was found to favour the woody debris where they were observed foraging and possibly using the structure to boost their predation success on other fish, while *P. afer* was found to favour the middle habitat where they were found schooling. Some of these habitats found within these pools may be viewed as critical habitats as they are essential to the survival of both *P. afer* and *S. capensis*. For example, the woody debris or fern root habitats may be critical for *P. afer* as they were recorded numerous times foraging within these habitats.

With an increase in threats to freshwater habitats, a focus needs to be placed on areas that are under serious threat or areas that contain various endemic fish species. The creation of a variety of artificial habitats should be explored to determine the specific habitat requirements for the respective species. Thereafter, a multidisciplinary approach (Dafforn *et al.*, 2015) could be used to create artificial habitats incorporating structural components that provide suitable habitat for different species.

The use of artificial habitats can be used as a possible conservation measure for freshwater fishes (Schlaepfer *et al.*, 2005; Yokomizo *et al.*, 2007; Westhoff *et al.*, 2013) to boost population numbers by providing habitats critical to their life histories. Fishes have been found to utilise artificial habitats whether in *situ* (Clark & Edwards, 1999; Pratt, 2005; Santos *et al.*, 2008) or in a laboratory setup (Kadye & Booth, 2014; Magellan & Garcìa-Berthou, 2015) for refuge or foraging. Similar results were found in this study with *S. capensis* making use of the artificial habitats for refuge and *P. afer* making use of the artificial habitats for foraging. This confirms that artificial habitats can be used to provide specific services to fish species, especially freshwater fishes that are facing population declines due to habitat modification or destruction.

In the case of *S. capensis*, artificial structures were highly successful in providing refugia. Behavioural observations also showed that *S. capensis* were regularly using the artificial structures for refugia often going into a pipe, swimming out, and going back into another pipe. *Sandelia capensis* is a cryptic species (Ellender *et al.*, 2012) that is more likely to be resting in an area as opposed to swimming around the pool. *Sandelia capensis* has also been found to feed on juvenile fishes (Marriott, 1998) and the use of complex structures within habitats could be due to trying to increase their predation success on other fishes within the pool (as ambush predators it is best for them to be in habitats that provide shelter, so they can hide easily). In this study, *S. capensis* were rarely observed in an open habitat, such as the middle or in faster flowing waters at the inflow or outflow. Although in this study it appears that the artificial structures constructed here were successful at providing refugia for *S. capensis,* it is difficult to say whether it was an overall success as more work needs to address different kinds of structures that provide different services to this species.

On the contrary, *P. afer* were not observed within the PVC pipes of the artificial habitats but were observed feeding off detritus which had settled on top of the pipes. This result is contradictory to those shown by Kadye and Booth (2014) who showed in a laboratory setting that *P. afer* preferred sheltered habitat (made from polyethylene strips). A possible cause for this difference is that one experiment was done *in situ* which observed *P. afer* in their natural setting as opposed to in a lab where they might be frightened and naturally choose a sheltered area. Like previous findings (see Chapter 3) *P. afer* favoured the middle habitat over any other habitat within the pool. Behavioural observations show that in the middle habitat they were mainly schooling or resting. On the contrary to *S. capensis*, *P. afer* may be utilising the middle of the pool where there are fewer or no complex structures to evade predators such as *S. capensis*. The highest behavioural counts were found in the fern root and woody debris areas. *Pseudobarbus afer* were found to be feeding there regularly as well as using these areas for refuge.

Although it was difficult from this study to determine whether the artificial habitats served a conservation purpose (due to only one kind of artificial habitat being constructed), they were highly utilised by *S. capensis*. This shows that future conservation geared towards protecting *S. capensis* can make use of artificial habitats if they provide areas of refuge. Artificial habitats in this study were constructed from white PVC piping, which may have been a deterrent within the first few days, but as soon as pipes were covered by detritus their colour was barely visible. However, future studies could make use of artificial structures that are different colours and, in this case, a dark-coloured artificial structure would have possibly been better as in would have blended in with the substrate of the pool. Future studies could include constructing artificial habitats that are aimed towards mimicking habitat types that are favoured by *P. afer*. This could include designing a habitat that is not rigid (possibly mimicking fern root) and replacing an already favoured habitat (such as the woody debris) with another tree-like structure. Such a study could also examine whether a habitat has become available for use by another species if one species is utilising the artificial habitat and another species is not. Lastly, it would be important to monitor the artificial structure at varying times of the day to assess whether diel patterns of the species in question, is affecting the utilisation of such a habitat.

This study showed that the use of action cameras *in situ* are a viable option for assessing habitat utilisation by two imperilled species in the presence of artificial structures. With the use of action cameras, it is possible to quantify habitat utilisation by calculation proportional occupancies of species within a certain habitat and make inferences on behaviour. This helps to identify why the habitats are being utilised by each species and can inform future studies on the use of artificially constructed habitats within headwater streams.

CHAPTER 5

GENERAL DISCUSSION AND RECOMMENDATIONS FOR FUTURE RESEARCH

5.1 General discussion

This thesis was made up of several studies addressing some of the first assessments of habitat use by the imperilled species *Pseudobarbus afer* and *Sandelia capensis*, and one of the few studies in South Africa that make use of action cameras to detect abundance of fish species in freshwater systems (others include Ellender *et al.*, 2012 and Weyl *et al.*, 2013). To date, such research has been limited due to technological limitations such as the ability of cameras, however there has been a recent emergence of high-quality action cameras that makes this work viable. Furthermore, very few studies have been conducted on the range restricted endemic fishes of South Africa (Wishart & Day, 2002). With the increase of human-mediated impacts on freshwater systems (Meybeck, 2003), threats to freshwater fishes are increasing (Vörösmarty *et al.*, 2010) and the monitoring of these threatened fish populations is required to determine appropriate conservation strategies.

One component of this thesis aimed to assess the effectiveness of videos and photographs derived from action cameras for determining the relative abundance of fishes within headwater streams, using snorkel surveys as a baseline method to determine abundance. This was done by assessing whether a 15-minute filming period was sufficient, after which, fish abundance, or density, estimates from videos and photographs were compared to abundances derived from snorkel surveys. Secondly, this thesis aimed to determine whether estimates derived from still photographs and videos were correlated to justify the use of photographs in preference to the more time-consuming analysis of videos. Photographs and videos were then used to assess the habitat utilisation of the two-focal species by quantifying abundance in each habitat and determining habitat utilisation through behavioural inferences following the introduction of an artificial structure as an alternate habitat.

5.1.1 Validation of filming time

Following recommendations from Ellender *et al.* (2012), an initial filming time of 15 minutes was chosen. Through the construction of accumulation curves, it was demonstrated that depending on camera placement, a minimum of four minutes was needed to detect 0.9 of the asymptotic mMaxN counts of *P. afer* in the simplest habitat with the lowest abundances. The maximum amount of time taken to reach a proportion of 0.9 of the asymptotic mMaxN of *P. afer* was 12.5 minutes in the middle of the pool where the largest portion of the population was present. This demonstrated that at least 12.5 minutes of filming time (plus a three minute acclimation time) be used and, therefore, the 15-minute filming time used here was adequate for detecting *P. afer* abundance.

5.1.2 Comparison of methods

It was found in Chapter 2 that the use of underwater video analysis (UWVA) was a viable method for detecting fish species abundance in the study headwater streams. Snorkel surveys were used as a baseline method for the absolute abundance of *P. afer* found within three pools in the Blindekloof tributary and seven pools within the Fernkloof tributary of the Swartkops River. The use of snorkel surveys has limitations that might skew counts of fish due to potential disruption of a snorkeler (Collins *et al.*, 1991; Beddow *et al.*, 1996; Cooke & Bunt, 2004) but is the best method of estimating absolute abundance of fish populations within a waterbody. Initially, the middle camera was used for analyses following recommendations from Ellender *et al.* (2012) and due to the high counts of fish detected from the middle camera placement in each pool compared to the other placements. However, when only the middle camera from each pool was used, estimates from UWVA and snorkel-survey estimates were found to be significantly different. Subsequent analyses, using multiple camera placements, showed that UWVA consistently yielded higher estimates of density and abundance than snorkel surveys. When multiple cameras were used, the slope derived from UWVA vs snorkel surveys did not differ significantly from 1:1 and, therefore, it was concluded that UWVA based on multiple cameras could be used in place of snorkel surveys.

5.1.3 Videos vs still photographs

It was demonstrated in Chapter 3 that estimates derived from the use of UWVA and still photographs were strongly correlated and that the slope of photographs vs videos did not differ significantly from a 1:1 relationship. This is an indication that both photographs and videos from action cameras are acceptable sampling techniques and that still photographs could be used in place of videos to reduce analysis time and storage requirements. The selection of the technique should, however, be based on the need of the sampler. For quantitative counts on fish species populations within a pool, still photographs are suitable. For inferences on behaviour, videos should be preferred (O'Neal, 2007). The information on behaviour provided to the sampler/researcher from still photographs is limited as there is no movement captured in a photograph. The sampler may be able to decipher why a fish species is using a certain habitat from a still photograph, but videos are better when making accurate deductions on fish behaviour. There were no significant differences found in proportional occupancies within habitats over the different visitations (all $p > 0.05$). Therefore, still photographs were used for quantitative abundance estimates for fish populations within headwater streams in Chapters 3 and 4 to evaluate habitat utilisation of the two-focal species. Videos were used in Chapters 3 and 4 to assess behaviour to explain the observed habitat utilisation.

5.1.4 Habitat utilisation

Habitat utilisation by a fish species is dependent on its requirements (Orpwood *et al.*, 2008) and stage in its life cycle (Schlosser, 1991). It was found (Chapter 3) that *Pseudobarbus afer* and *Sandelia capensis* favoured different habitats within four pools of the Fernkloof tributary of the Swartkops River. *Pseudobarbus afer* preferred congregating in the middle of the pool occasionally schooling while *S. capensis* preferred more structure-orientated habitats, such as woody debris and fern root. Schooling behaviour exhibited by *P. afer* is likely due to a need for rapid transference of information within the school, such as the presence of a predator (Brown & Laland, 2011). The major aquatic predator present in the pools sampled is the nocturnal anguillid eel *Anguilla mossambica*. The reason that the diurnal *P. afer* still exhibit schooling can be attributed to the genetic predisposition of a species which tends to hide from predators naturally (Gornman, 1988). Furthermore, the typical schooling behaviour exhibited by *P. afer* in the middle habitat could be attributed to avoiding predation by the structure-orientated predator *S. capensis,* which are more likely to be found in more complex habitats and not the open middle. High proportions of *P. afer* found within woody debris and fern root are attributable to their foraging techniques and the putative use of these structures to avoid avian predators such as kingfishers and herons. *Pseudobarbus afer* feed on organisms within detritic material and, as such, are likely to occur in habitats that are covered in detritus or decomposing leaf matter.

Relatively low numbers of both *P. afer* and *S. capensis* were found in the shallow fast-flowing waters of the inflow and outflow habitats. *Pseudobarbus afer* were likely to avoid these habitats as they offer no benefit. The water flows too fast for detritic material to collect, the water is shallow and minimal refugia is available. *Sandelia capensis* were probably absent from these areas as the water was shallow and there was limited structure or habitat complexity in both the inflow and outflow habitats. From Chapter 3, it was evident that there was a wide array of habitats in each pool where *P. afer* and *S. capensis* could coexist due to their different needs and different habitat associations. It was also evident that the observation alone on habitat utilisation was not enough to provide information on a species' habitat associations and behavioural observations were required. Finally, it was also evident that a habitat could be artificially recreated and may be utilised by the two-focal species. The findings of Chapter 3 were contrary to the lab-based findings of Kadye & Booth (2014), who found that *P. afer* was likely to favour cryptic artificial habitat (made from polyethylene strips) over the middle section. However, this study was conducted in a laboratory and this highlights the importance of conducting such experiments *in situ*.

5.1.5 Artificial habitat utilisation

Some habitats within freshwater systems may be termed as critical and essential to the survival of a species (Rosenfeld & Hatfield, 2006). Understanding where these habitats occur and whether they can be artificially replicated to enhance conservation measures is crucial (Nash *et al.*, 1999; Knaepkens *et al.*, 2004). In Chapter 4, artificial habitats were placed into the four pools monitored in Chapter 3 such that they did not overlap with the habitats previously monitored in the pool. All six habitats were then monitored in accordance with the methods developed in Chapter 3. A one-month acclimation period was included to allow fishes to become familiar with the introduction of foreign object. After the acclimation period, it was found that all four artificial habitats had been rapidly colonised by *S. capensis* while *P. afer* avoided using the pipes. One determinant for the success rate of an artificial structure is the colonisation rate by fishes (Pratt, 1994) and, in terms of this study, the artificial structures could be viewed as being successful for *S. capensis*. This rapid colonisation by *S. capensis* could be attributed to their preference for structured habitats that provide refuge.

Steps followed in Chapter 4 were like those followed of Santos *et al.* (2008) who evaluated the use of artificial structures within a neotropical reservoir using a pyramid structure constructed of PVC piping and included numerous post-deployment visits to each of the artificial structures. Like Santos *et al.*, (2008), data collection for Chapter 4 made use of an acclimation period to allow fish to become used to the artificial structure as well as there were many post-deployment visitations. Although *S. capensis* were found in each artificial habitat, there were still high numbers of *S. capensis* present in woody debris and fern root areas. This may be attributed to intra-species competition between *S. capensis* individuals. *Pseudobarbus afer* were classified as using artificial habitats because they were observed picking at detritus material that had settled on all artificial structures however, they were not found inside any of the pipe structures throughout the study.

5.1.6 Main findings

The research conducted in this thesis has increased our understanding of the habitat utilisation by two endemic species *P. afer* and *S. capensis*, currently impacted by multiple anthropogenic stressors (Ellender *et al.*, 2015) such as habitat degradation through pollution, over abstraction of water and flow modification, in addition to the presence of alien or invasive fish species. Understanding the threats to these species, and their habitat utilisation, is important for their conservation (Ellender *et al.*, 2015) and will allow authorities and researchers to be better informed on how to conserve them. Assessing habitat utilisation by the two species was done with the least destructive methods (videos and still photographs) and this made this study viable. If destructive or invasive methods were used, the study might not have been permitted due to the threatened status of both fish species.

It was shown that relative abundance estimates derived from the use of videos and photographs were strongly correlated to those derived from snorkel surveys indicating that action cameras could be used in place of snorkel-based sampling techniques. Other conventional sampling techniques, such as netting or electrofishing, are potentially harmful to the focal species and should be avoided when assessing abundances of imperilled fish species.

A better understanding of both these species has brought to light areas that need to ensure there is not a rapid depletion of population numbers due to human-mediated impacts on headwater streams (Ellender *et al.*, 2011). Weyl *et al.* (2014) showed that continuous monitoring and identification of critical habitats utilised by *Pseudobarbus* species is crucial for the conservation of this species. It is, therefore, important that future studies be built on the foundations provided by this thesis to further expand our knowledge of the endemic freshwater fishes of South Africa and to explore the use of action cameras further in the monitoring of imperilled fishes and exploring their habitat utilisation.

5.2 Recommendations for future research

Based on the findings presented in this thesis, the following priorities were identified for future research. Firstly, comprehensive studies of habitat utilisation need to be carried out for other imperilled fish species within South Africa. This particularly holds true for areas that are high in endemism and threatened species; such as the Cape Fold Ecoregion. Understanding habitat utilisation will provide information on the status of specific species and help identify which areas should be priorities in terms of conserving habitats. Future research should also aim to assess all habitats as found within a waterbody to avoid excluding any species or behavioural observations.

With respect to the use of action cameras to document habitat use by various fish species, future research should evaluate the minimum number of cameras needed to record the maximum abundance of fishes within a waterbody. The minimum number of action cameras needed may vary according to the size and/or depth of the pools surveyed. Furthermore, the use of baited versus unbaited cameras should be evaluated to determine whether baiting cameras results in the detection of higher abundances of fishes. Baited cameras are popular in marine systems for detecting the maximum number of fish species within an area but have not been extensively researched in freshwaters. Lastly, the optimal acclimation time for action cameras should be evaluated to determine the most effective filming time to detect maximum abundances of fish species. The acclimation time may vary between species and with the characteristics of the river system being surveyed.

For artificial structures being used as habitats, future habitat utilisation research should investigate replicating natural habitats or constructing habitats found within the river systems; such as artificial woody debris. This could include the construction of composite habitats consisting of multiple habitat components that can be used by multiple species within a waterbody. Furthermore, researchers could assess whether artificial structures made from a more natural colour are more suitable than those made from colours not found in the pool. Information gained from exploring artificial structures could aid in environmental rehabilitation projects for certain severely threatened fish populations (Santos *et al.*, 2008), particularly in areas where habitat modification and degradation are the primary threat to the fish species.

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