



Detecting Native Freshwater Fishes Using Novel Non-invasive Methods

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Improving the ability to detect and quantify rare freshwater fishes in remote locations is of growing conservation concern, as the distributions of many native fishes are contracting to such locations where there are reduced anthropogenic and invasive species pressures. However, conventional sampling methods, e.g., backpack electrofishing and seines, tend to be heavy and bulky, thereby making them difficult to transport into remote areas with no road access. These conventional sampling methods also require physical handling of fishes, which may cause stress, harm, and mortality—all undesirable side effects for rare fishes. Thus, visual observation methods, such as underwater camera and snorkel surveys, and environmental DNA (eDNA), that are easily transportable and do not require physical handling of fishes, are being more frequently used in freshwater ecosystems. However, there have been few studies on the relative effectiveness of these three methods for detecting and quantifying freshwater fishes. In this study, the species-specific detection probabilities between the three methods, and abundance estimates derived from the visual observation methods were compared, and their utility for sampling rare fishes in remote locations in South Africa was evaluated. Underwater cameras and snorkel surveys detected slightly different species within a fish community. For the redfins, the detection probability using underwater cameras (0.96, SD = 0.03) was highest, followed by snorkel surveys (0.93, SD = 0.05), and eDNA (0.70, SD = 0.21). The visual observation methods were positively correlated with pool length, while eDNA was negatively correlated with turbidity. For Cape Kurper, the detection probability using underwater cameras (0.75, SD = 0.15) was highest, followed by snorkel surveys (0.68, SD = 0.16), and eDNA (0.64, SD = 0.19); all three methods were negatively affected by water turbidity. It is recommended that decisions on which sampling method to use in remote locations should depend on whether the study requires population- or community-level information, spatial scale required, and resource availability, as each method has its own strengths and weaknesses. Generally, eDNA is the most expensive method and requires specialized facilities and equipment, while underwater cameras require video analyses that are more time consuming to analyze than snorkel surveys.

Keywords: underwater cameras, snorkel surveys, eDNA, streams, endangered fishes, South Africa, detection probability

INTRODUCTION

Imperfect detection of species biases our ability to quantify community richness and population abundances (Guillera-Arroita, 2017). The magnitude of this imperfect detection varies based on the rarity and behavior of a species, while our ability to detect the species will vary based on sampling methods (MacKenzie et al., 2018). This is further complicated when the focal species are endangered as most conventional sampling methods require capture and physical handling of animals, inducing stress on and, at times, causing mortality of, target species and by-catch (Putman, 1995; Romero, 2004; Ellender et al., 2016). This is especially true when sampling and monitoring freshwater fishes. For example, electrofishing can have a mortality rate of 3–10% and cause other injuries, such as spinal fractures, that would affect the fish's ability to survive (Snyder, 2003; Dolan and Miranda, 2004). A 3% mortality rate may be acceptable for widely distributed fishes with large population sizes but may have a population-level effect on endangered species with restricted distribution ranges. In addition, the distributions of many endangered fishes have been contracting into headwater areas, into refuges from invasive species and other anthropogenic disturbances that have yet to reach (Lowe and Likens, 2005; Colvin et al., 2019). In many places, headwater refuges are in remote locations with little to no road access where bulky conventional sampling equipment, such as backpack electrofishers and seine nets, may be impossible to transport (Thurow et al., 2012; Ebner et al., 2015). Therefore, readily-transportable, non-harmful sampling techniques are required to offset stress and injury to endangered fishes and by-catch in remote areas (Costello et al., 2016). In freshwater systems, visual observation and environmental DNA (eDNA) are increasingly popular non-invasive sampling methods (Janosik and Johnston, 2015; Struthers et al., 2015). Visual observation techniques can be undertaken using underwater cameras or snorkeling (Ellender et al., 2012; Struthers et al., 2015) and eDNA tests for the DNA of organisms in water samples to assess species presence (Lacoursière-Roussel et al., 2016a).

Underwater camera recordings are frequently used in freshwater and marine ecosystems (e.g., Struthers et al., 2015; King et al., 2018; Castañeda et al., 2020); this method has many advantages and some disadvantages (Mallet and Pelletier, 2014). Underwater cameras can be used to estimate relative abundance and species richness and study the behavior of fishes in their natural environment but require clear water to do so. Recordings can be stored and verified by other investigators. However, cameras require accessories for power and memory (Friesen and Chivers, 2006; Thurow et al., 2012; Assis et al., 2013; Ebner and Morgan, 2013; Domenici et al., 2014; Mallet and Pelletier, 2014; Struthers et al., 2015). If electricity is not accessible, as in many remote areas, extra batteries or alternate power sources (e.g., generators, solar panels) and memory cards are required. These specialized electronics may be expensive or unfeasible to purchase for some researchers working in remote areas; hence, snorkeling surveys may be better suited in these situations.

Snorkeling is another visual observation method that has been used to detect and estimate fish densities in small headwater

streams (Weyl et al., 2013; Chamberland et al., 2014; Ellender et al., 2018). Snorkel surveys generally outperform seining and electrofishing, except when sampling cryptic species (Brock, 1982; Jordan et al., 2008; Saunders et al., 2011). Generally, snorkel surveys tend to be the most simple and cost-efficient method for observing fishes, as it requires very little equipment. Information on community composition, population size structure, and habitat use can be collected using snorkel surveys (Thurow and Schill, 1996; Ebner et al., 2015; Macnaughton et al., 2015). However, snorkel surveys could be limited by water depth, water clarity, and snorkeler training and experience, factors that could reduce consistency in, and reproducibility of, the data collected (Thurow et al., 2012). In marine environments, there has been much debate as to whether underwater cameras or snorkel surveys collect the most accurate and precise data on fish populations and communities (Langlois et al., 2010; Pelletier et al., 2010; Watson et al., 2010); however, comparative studies between the two visual observation methods are rare in freshwater habitats (Ebner et al., 2015). Although underwater cameras and snorkel surveys are becoming more widely used in freshwater ecology, we know little about the relative efficiencies of detecting rare fishes.

Environmental DNA is an increasingly used non-invasive method for surveilling invasive and endangered fishes in freshwater ecosystems. eDNA protocols concentrate DNA molecules that have been shed into their local environment (e.g., water, soil, snow, air) to detectable levels using PCR (Ficetola et al., 2008; Thomsen and Willerslev, 2015). PCR-based assays may target individual species with high specificity (Baker et al., 2018; Fernández et al., 2018; Mauvisseau et al., 2019), or facilitate the holistic screening of entire biotic communities through the massively parallel DNA sequencing of diagnostic genetic markers (Harper et al., 2018), and have made it possible to detect DNA from water samples even at very low quantities (Ficetola et al., 2008; Thomsen and Willerslev, 2015; Harper et al., 2018). However, the high sensitivity of eDNA increases the risk of false positives from contamination, requiring specialized tools for collecting water samples in the field and a clean laboratory set up for extracting DNA separate from the PCR/sequencing facility (Goldberg et al., 2016). Despite these costly hurdles, the economies of scale associated with a high-throughput method like eDNA has led to its application in many large-scale monitoring projects (Jerde et al., 2013; Biggs et al., 2015; Balasingham et al., 2018). However, the effectiveness of eDNA studies may depend on the hydrological and physical aspects of the habitat (Jane et al., 2015; Balasingham et al., 2017; Harper et al., 2018), and species-specific differences in shedding, behavior, and abundance (Thomsen and Willerslev, 2015). In addition, eDNA studies have predominantly focused on temperate species, and the utility of these studies in warmer tropical and subtropical conditions may differ (Eichmiller et al., 2016; Cantera et al., 2019; Doble et al., 2019).

The purpose of this study is to compare the detection probabilities and abundance estimates of the two visual observation methods and eDNA for fish communities in remote locations and to assess which method is best suited in different situations. The study focuses on the Eastern Cape

Redfin (*Pseudobarbus afer*), Gamtoos River Redfin (*Pseudobarbus swartzi*), and Cape Kurper (*Sandelia capensis*) in a remote area of Eastern Cape Province of South Africa. Eastern Cape Redfin is listed as Endangered by IUCN (Chakona et al., 2017a), the closely related Gamtoos River Redfin is listed as Vulnerable by IUCN (Chakona et al., 2017b), and Cape Kurper is Data Deficient but has a declining population trend (Chakona, 2018). We hypothesize that the detection probabilities of all three methods will decrease with increasing spatial scale of the pools sampled within the streams (length, width, depth) and with increasing water turbidity.

MATERIALS AND METHODS

Study System

The study was conducted in four clear headwater streams in Eastern Cape Province, South Africa typical of headwater stream refugia for native fishes in the eastern part of the Cape Fold Ecoregion (Ellender et al., 2017). Three streams, Fernkloof, Waterkloof, Blindekloof, tributaries to the Swartkops River, are within the Groendal Wilderness Area (Figure 1A) and the Bos River tributary of the Kouga River which runs in part through the Skilderkrantz Private Nature Reserve (Figure 1B). Both the Skilderkrantz Private Nature Reserve and the Groendal Wilderness Area are within the Baviaanskloof Mega Reserve and are part of the Cape Floral Region Protected Areas World Heritage Site (UNESCO, 2015). The tributaries run through mountainous areas, bounded by indigenous forest, with few roads or maintained trails to provide access to them. Reaching the sampling sites requires hours of hiking through rugged terrain and wading and swimming through pools (Figure 2). Both watersheds have low native freshwater fish species richness (Ellender et al., 2017). Fish faunas contain African Longfin Eel (*Anguilla mossambica*), Cape Kurper, Goldie Barb (*Enteromius pallidus*), and River Goby (*Glossogobius callidus*), Eastern Cape Redfin and Gamtoos River Redfin (Supplementary Table 1) (Chakona and Skelton, 2017; Ellender et al., 2017). Both watersheds have had intentional and unintentional introduction and establishment of non-native fishes. Non-native faunas include Common Carp (*Cyprinus carpio*), African Sharptooth Catfish (*Clarias gariepinus*), Largemouth Bass (*Micropterus salmoides*), Smallmouth Bass (*Micropterus dolomieu*), Spotted Bass (*Micropterus punctulatus*), and Banded Tilapia (*Tilapia sparmanii*) (Supplementary Table 1) (Ellender et al., 2011). Because Eastern Cape Redfin and Gamtoos River Redfin were, until recently (Chakona and Skelton, 2017), considered the same species within the *Pseudobarbus afer* complex throughout the sampling period and exhibit very similar ecologies, the data from the two species were combined in the study and termed redfins.

Data Collection

In February and March 2015, sampling for freshwater fishes using cameras, snorkel, and eDNA snorkel surveys was conducted in isolated pools along the length of the streams. For cameras and snorkel surveys, 23 pools were sampled spanning 8 km in the Bos River, 4 pools spanning 3 km in the Kouga River, 13 pools spanning 3 km in the Fernkloof, 10 pools spanning 3 km in

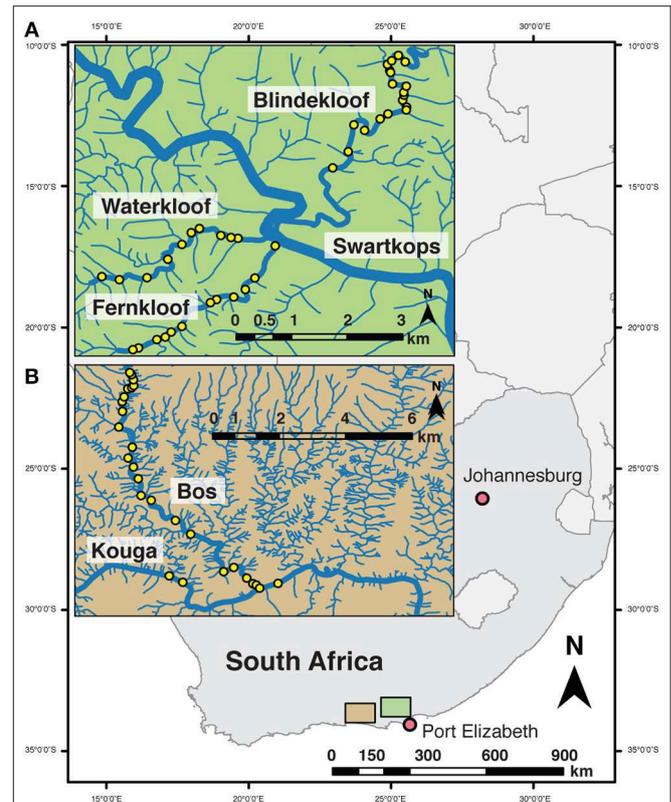


FIGURE 1 | Location of sampling sites (yellow points) across two watersheds in Eastern Cape, South Africa; **(A)** Swartkops watershed within the Groendal Nature Reserve; and **(B)** Kouga watershed, Bos River tributary within the Skilderkrantz Nature Reserve.

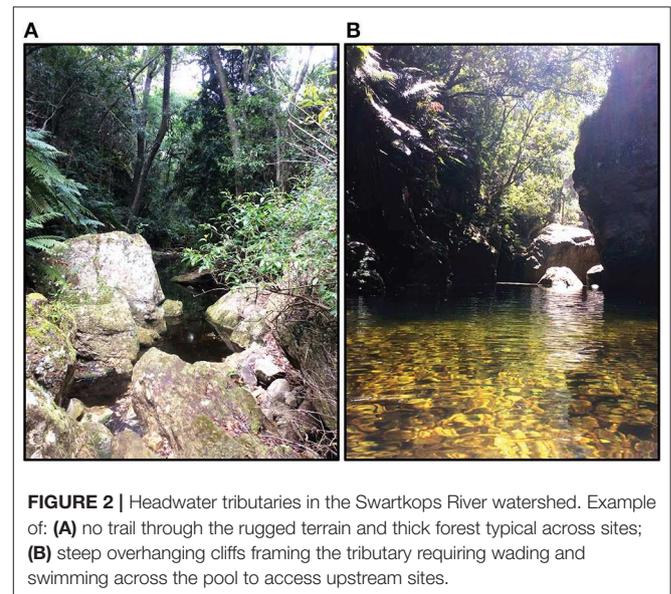


FIGURE 2 | Headwater tributaries in the Swartkops River watershed. Example of: **(A)** no trail through the rugged terrain and thick forest typical across sites; **(B)** steep overhanging cliffs framing the tributary requiring wading and swimming across the pool to access upstream sites.

the Waterkloof, and 19 pools spanning 5 km in the Blindekloof (Figure 1). For eDNA, water samples were taken at seven sites in the Bos River, three sites in the Kouga, four sites in the Fernkloof,

four sites in the Waterkloof, and four sites in the Blindekloof, which were all a minimum of 500 m apart.

Water samples for eDNA analysis were taken before any field personnel waded through the stream and before deploying other sampling gear to prevent contamination from field clothing and equipment that had been in contact with stream water downstream. Three 2-L water samples were taken within 5 cm of the surface of each pool using pre-sterilized Nalgene polycarbonate plastic collection bottles. Sterilization of the collection bottles and carrying dry-bags was done at the field station before sampling by soaking them in a 10% bleach solution (of 6% w/v sodium hypochlorite) for at least 10 min, then air dried. All collected water samples were packed in ice and after three or four pools were sampled, they were carried immediately downstream to the field station for filtration. All eDNA water samples were collected in <5 field days.

Upon returning from the sampling area, water samples were filtered on the same day at the field station in a room dedicated to filtration that was cool and sterilized. A vacuum pump and tripartite manifold filtration system were used to filter the water samples through glass microfiber filter papers [47 mm diameter, 1.2 μm pore size (VWR 696-filter, product No: 28333-139)]. Sterile forceps were used to manipulate the filter papers onto the funnels and into microcentrifuge tubes. Approximately half of the water was processed per filter paper resulting in two filter papers per water sample, which were stored in separate microcentrifuge tubes and filled with molecular grade ethanol (> 95%). The process was repeated for each water sample where all equipment was sterilized and dried between samples. Controls were conducted using the same procedure with distilled water between sample runs.

eDNA samples and the environmental controls were extracted in a UV cabinet in a building separate from the facility used for PCR using a modified DNeasy Blood & Tissue Kit (Qiagen) protocol designed for eDNA samples (Goldberg et al., 2011; Lacoursière-Roussel et al., 2016b). DNA concentrations were taken for a subset of the eDNA samples, environmental controls, and extraction controls using a Qubit 4 Fluorometer (Invitrogen) to ensure extraction success.

Forward and reverse primers were designed to target a < 200 bp region of the *cytochrome b* mitochondrial gene of both redfins (*P. afer* and *P. swartzi*) and Cape Kurper (*S. capensis*) ensuring at least 6 nucleotides differ between the target species and other co-occurring species in the localities sampled (Supplementary Table 1). The specificity of these primers (Supplementary Table 2) for solely the target species was tested *in vitro* using conventional PCR with genomic DNA from each target species and other co-occurring species as the template. Genomic DNA was extracted from tissue samples preserved in ethanol extracted using a DNeasy Blood & Tissue Kit (Qiagen). Each PCR reaction contained 6 μL of 2X Phusion Hot Start II High-Fidelity DNA Polymerase, 600 nM of forward and reverse primers, 2 μL of template DNA, and ddH₂O for a final volume of 12 μL . Following an initial denaturation phase at 95°C for 3 min, DNA was amplified for 40 cycles of 98°C (20 s), 60°C (15 s), and 72°C (15 s), followed by a 5 min extension phase at 72°C. PCR products were visualized on a 2% agarose gel

and were compared to a 100 bp ladder to check that amplicons were the correct size. At least one amplicon from each watershed was purified and sequenced using Sanger sequencing to further ensure the specificity of each primer pair.

All eDNA samples, environmental controls, and extraction controls were tested for the presence of redfins and Cape Kurper using quantitative PCR (qPCR). Each qPCR reaction contained 10 μL of Power SYBR Green Master mix (Applied Biosystems), 200 nM of the species-specific forward and reverse primers (Supplementary Table 2), 4 μL of template (diluted in ddH₂O 1:10), and ddH₂O for a final volume of 20 μL . Each sample was assayed in triplicate on a 96 well plate alongside at least three no template controls (NTCs) containing ddH₂O instead of template. All plates were analyzed on a QuantStudio™ 3 Real-Time PCR System (Applied Biosystems). Thermocycler conditions were set to the manufacturer's defaults (50°C for 2 min; 95°C for 2 min; followed by 40 cycles of a 15 s 95°C denaturation step and an annealing/extension step of 1 min at 60°C). C_q values, a measure of when the fluorescent signal passes the threshold for detection, were calculated using the second derivative method. Amplification of the correct target sequence and absence of primer-dimers was confirmed by a comparison of a melt-curve assay (from 60 to 95°C). Positive sequence identity of the qPCR products was also confirmed with a BLAST search of the Genbank nucleotide database with query sequences from a subset of PCR products sequenced at the TCAG sequencing facility at the Hospital for Sick Children, Toronto. The limit of detection (LOD) and the efficiency of the qPCR reaction was determined using a standard curve, as MIQE guidelines [Minimum Information for the Publication of Quantitative PCR Experiments (Bustin et al., 2009)], generated using 10-fold dilutions of genomic DNA from 1 to 1 $\times 10^{-6}$ ng/ μL for *P. swartzi* and 10 to 1 $\times 10^{-6}$ ng/ μL for *S. capensis*. A positive hit for each target species in the eDNA and control samples was assigned to a sampling site if the fluorescent signal of any of the three technical replicates surpassed the threshold for detection and if melt temperatures were within the range of temperatures observed in analysis of genomic DNA samples (78–79°C for the *S. capensis* and 75–76°C for *P. swartzi*). We removed any eDNA samples from the analysis if any hits were detected in an environmental control taken from the same site.

After collecting eDNA samples, a GoPRO (Hero4 Black) was mounted on a flexible tripod (GorillaPod) and placed at the end of each pool facing upstream. The camera recorded video for 30 min (video resolution 1080p, 30 frames per second, ultra-wide frame of view, and 1,920 \times 1,080 screen resolution) (Ellender et al., 2012). A 30 min recording is standard for monitoring native fish populations in South African streams and rivers and is sufficient to achieve the highest MaxN (Ellender et al., 2012; Weyl et al., 2013). In the lab, all videos were viewed, and the maximum number of adult and juvenile fishes was recorded for every 30 s time frame and scored as a positive detection. MaxN was used as the measure of relative abundance (Campbell et al., 2015), in which the single highest count of a target in the specific recording is retained.

Upon retrieval of the camera, a two-pass snorkel survey was conducted where the snorkeler started at the downstream end

of the pool and zigzagged upstream (to maximize sampling all microhabitats across a pool) until they reached the end of the pool and repeated the same zigzagging pattern back downstream (Ellender et al., 2011). All fishes observed were identified to species and counted. The counts of the two passes were averaged and converted to density (fish m^{-2}), by dividing the average count by the surface area [length (m) \times average width (m)] of the sampled site (Ellender et al., 2012; Weyl et al., 2013).

At each pool, several habitat variables, hypothesized to affect the occupancy of the fishes and detection probability of the different gear types, were measured. Total length (m) of the pool was measured; then the pool was separated in 5–6 transects of equal length, and the width (m) of each transect measured. At each transect, depth (m) and the dominant sediment type, was recorded at 0.2 m from the waterline on each side and at the midpoint of the stream, totaling three sampling points per transect (Ellender et al., 2012). Water turbidity (nephelometric turbidity units–NTU) was measured using a Hanna HI 98703 turbidimeter (HANNA Instruments Inc., Woonsocket, USA).

Statistical Analysis

Multi-scale, single-season occupancy models were built to calculate the detection probabilities of each gear type for the native and non-native fishes (Nichols et al., 2008; MacKenzie et al., 2018). The multi-scale, occupancy-model approach estimates the detection probability of different gear types (p) deployed during the sampling period, probability of occupancy (ψ), and probability that the species is present during the sampling (θ) (Nichols et al., 2008). The top occupancy model was determined by holding detection probability constant and testing the different habitat covariates on occupancy; the model with the lowest AIC was used to determine detection probabilities of each gear type. Using the top occupancy model, detection probability was modeled as constant across sampling methods, different across methods, and covarying with average pool depth (m), average pool width (m), pool length (m), surface area (m^2) [length (m) \times average width (m)], volume (m^3) [length (m) \times average width (m) \times average depth (m)], and turbidity (NTU). Correlated variables were not included in the same model. We hypothesized that an increase in any of these spatial covariates would negatively affect the detection probability of each method, as cameras and snorkelers have a limited field of view (Thurow et al., 2012), and DNA can be diluted in larger water bodies (Jane et al., 2015). Further, we hypothesized that turbidity would have a negative effect on the detection probabilities of all three methods, as an increase in turbidity would reduce visibility, a requirement for visual observation methods (Thurow et al., 2012), and turbidity, which can be a proxy for PCR inhibitors present in the water, can decrease detection by eDNA (Jane et al., 2015). For camera detections, if a fish was seen at least once in a video recording it was scored as 1; for snorkel surveys, if the fish was seen in at least one of the two passes then it was scored as 1; and, for eDNA if there was one positive hit in one of the three water samples taken then it was scored as 1. To determine if three water samples are needed per pool, the same occupancy models were re-run with the three detections, e.g., if two of the three water samples had a hit, then it was scored as 1, 1, 0. Using the program PRESENCE, models were

run to calculate maximum likelihoods, and model selection was performed using AIC (Hines and MacKenzie, 2018). The model-averaged estimates were also calculated to obtain an overall detection probability for all three methods (MacKenzie et al., 2018). To assess the goodness of fit of our occupancy models, we attained the overdispersion parameter (c) by using Pearson chi-square statistics and parametric bootstrap test by running 10,000 bootstraps for the most global model (MacKenzie and Bailey, 2004). For models that were underdispersed ($c < 1$), $c = 1$ was set, and, for overdispersed models ($c > 1$), quasi-AIC (QAIC) was used, and AICc was used to correct for small sample sizes (Burnham and Anderson, 2002).

To test whether there is a correlation between the abundance estimates collected using underwater cameras and snorkel surveys, Spearman correlation tests were used due to the non-parametric nature of the abundance data. Data normality was tested using the “olsrr” package in R (Hebbali, 2018; R Core Team, 2019). Correlation tests were run between underwater camera MaxN and snorkel averaged count data, and underwater camera MaxN and snorkel density (individuals m^{-2}) (Ellender et al., 2012; R Core Team, 2019). To run linear regressions, the abundance data were $\log_{10}(x + 1)$ transformed to normalize the distribution of the residuals. To investigate the variation in habitat characteristics of the pools and fish densities between the tributaries, a Kruskal-Wallis test followed by Dunn’s *post-hoc* test was used (Dinno, 2017).

RESULTS

Fish Community Detection and Species-Specific Detection Probabilities Vary Between Methods

The species-specific qPCR primers designed for this study were specific for the target species and had good efficiency scores (102%) with a LOD of 1×10^{-4} ng/ μ L for *P. swartzi* and 1×10^{-5} ng/ μ L for *S. capensis* (Supplementary Figure 1). The eDNA analysis detected *Pseudobarbus* spp. in 14 of 22 sampling sites and *S. capensis* in 10 of 22 sites and was generally consistent across technical and biological replicates (Supplementary Table 3).

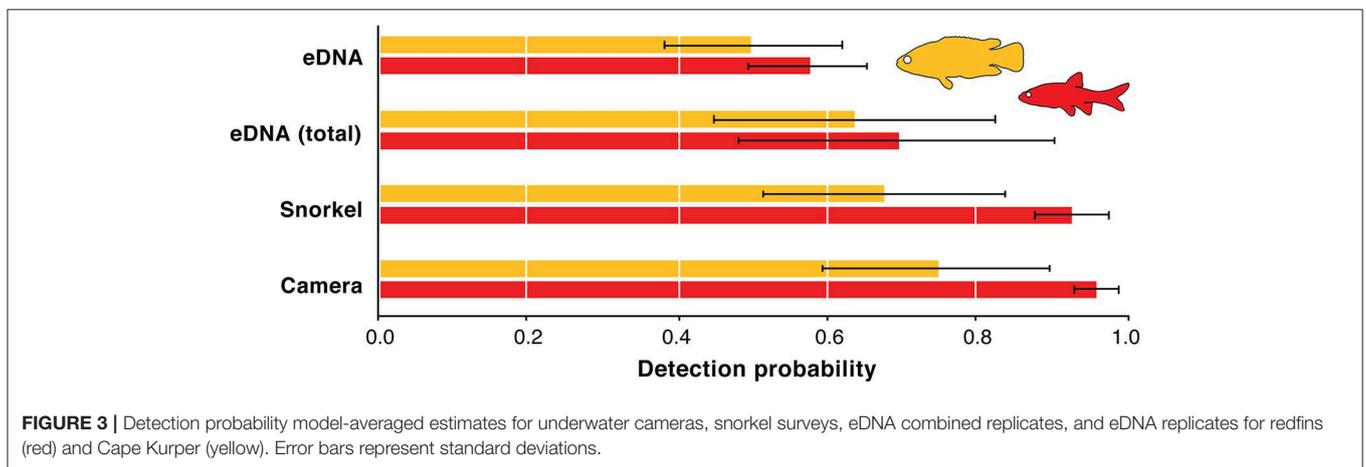
The raw detection data revealed that the visual observation methods detected most of the fish species, although there were slight, but not significant, differences between methods. Generally, underwater cameras slightly outperformed snorkel surveys for all fish species except for Goldie Barb and River Goby (Table 1). The cameras detected Goldie Barb 75% of the time while snorkel surveys detected it 81% of the time, and River Goby was detected by these methods 82 and 100% of time, respectively. These percentages are overestimates of detection as we cannot confirm that a species was present in a pool if it was not detected by either visual observation method, i.e., imperfect detection was not being considered as there were too few encounters to run the occupancy models. Underwater camera was the only method that detected African Longfin Eel and African Sharp-tooth Catfish.

Estimating the detection probabilities of the three methods was done only for redfins and Cape Kurper as there was

TABLE 1 | Number of detections of each fish species by underwater cameras (cam) and snorkel surveys (snork), and eDNA across sampled sites.

Species	Total of 69 sites sampled			Total of 22 sites sampled			
	cam	snork	Pools with fish presence	cam	snork	eDNA	Pools with fish presence
Redfins (<i>Pseudobarbus afer</i> , <i>Pseudobarbus swartzii</i>)	50	48	51	16	16	14	16
Goldie barb (<i>Enteromius pallidus</i>)	12	13	16	–	–	–	–
Cape kurper (<i>Sandelia capensis</i>)	34	32	36	10	10	10	11
River goby (<i>Glossogobius callidus</i>)	14	17	17	–	–	–	–
Smallmouth bass (<i>Micropterus dolomieu</i>)	5	4	5	–	–	–	–
Spotted bass (<i>Micropterus punctulatus</i>)	7	7	7	–	–	–	–
Banded Tilapia (<i>Tilapia sparmanii</i>)	12	11	13	–	–	–	–
African longfin eel (<i>Anguilla mossambica</i>)	1	0	1	–	–	–	–
African sharptooth catfish (<i>Clarias gariepinus</i>)	1	0	1	–	–	–	–

cam, underwater camera; snork, snorkel survey; eDNA, environmental DNA; –, no sampling.

**FIGURE 3** | Detection probability model-averaged estimates for underwater cameras, snorkel surveys, eDNA combined replicates, and eDNA replicates for redfins (red) and Cape Kurper (yellow). Error bars represent standard deviations.

an insufficient number of detections of the other fish species across the sampling area. Across the 69 sampling sites, the model-averaged detection probability estimates for the redfins was 0.96 (SD = 0.03) using underwater cameras, and 0.93 (SD = 0.05) using snorkel surveys, 0.70 (SD = 0.21) for combined-replicate eDNA, and 0.58 (SD = 0.08) for eDNA replicates (Figure 3). For Cape Kurper, the model-averaged estimates of the detection probability were 0.75 (SD = 0.15) using underwater cameras, 0.67 (SD = 0.16) for snorkel surveys, 0.64 (SD = 0.19) for combined-replicate eDNA, and 0.50 (SD = 0.12) for eDNA replicates (Figure 3). For both fishes, eDNA consistently underperformed while underwater cameras had the highest detection probability.

Habitat Variables Affect the Detection Probabilities of the Three Different Methods

To investigate the effect of habitat variables on each sampling method, multi-scale occupancy models were run with covariates hypothesized to affect each method individually and simultaneously while using the top covariate for occupancy. The covariates for the probability of occupancy and detection were pool length, average pool depth and width, surface area, and volume; additional covariates tested for occupancy included number of invasive fish species present and average and dominant sediment size. Further, turbidity was used as a covariate for detection probability.

TABLE 2 | Summary of the model selection using AIC for the occupancy and detection probability of redfins using underwater cameras, snorkel surveys, and combined eDNA.

Model ψ (avg width), θ (.)	AICc	Δ_i	w_i	K	-2Log(L)
p (camera&snorkel + length, eDNA(turb))	128.50	0.00	0.12	8	110.1
p (m + length)	128.70	0.20	0.11	7	112.86
p (camera&snorkel + surface area, eDNA(turb))	129.75	1.25	0.07	8	111.35
p (camera&snorkel + length, eDNA(.))	129.99	1.49	0.06	7	114.15
p (camera&snorkel + length, eDNA(avg depth))	131.06	2.56	0.03	8	112.66

Top five models are presented.

(.), constant detection probability across sites; m and &, methods-specific detection probability; avg, average; +, covariate effects both methods similarly.

There were four models with a difference in AICc value of <2 for redfins; however, the top model had a higher weight ($w_i = 0.12$) while the other five models had very similar log-likelihood values, indicating little evidence for their support (Table 2; Burnham and Anderson, 2002). The top model revealed that the detection probabilities (p) of underwater cameras and snorkel surveys differed but were positively influenced by increasing pool length (Figure 4A). eDNA detection probability decreased with turbidity (Figure 4B). The top occupancy model included the negative affect of pool width on the probability of occupancy (ψ) of redfins.

For Cape Kurper, there were four supported models within 2 AICc values of the top, where the top model had the higher weight ($w_i = 0.24$), while the other models had more parameters and similar log likelihoods, indicating little evidence for their support (Table 3). Based on the top model, the detection probabilities of underwater cameras, snorkel surveys, and eDNA differed, but were negatively affected with increasing turbidity (Figure 4C). The top occupancy model for Cape Kurper revealed a negative relationship between average pool width and probability of occupancy (ψ).

Strong Correlation Between MaxN and Snorkel Densities

There were significant strong, positive correlations between the abundance estimates collected by both methods for both fish species. For redfins, the Spearman correlation was 0.63 ($p < 0.001$) between MaxN and averaged snorkel counts and 0.83 ($p < 0.001$) between MaxN and the snorkel density. For Cape Kurper, the Spearman correlation was 0.83 ($p < 0.001$, Figure 5A) between MaxN and average snorkel counts and 0.85 ($p < 0.001$, Figure 5B) between MaxN and snorkel density. The log-transformation normalized the distribution of the residuals for the abundance data of redfins but not Cape Kurper. Therefore, linear regressions were only run for redfins, which had significant positive relationships between MaxN and snorkel averaged counts ($r^2 = 0.65$, $p < 0.001$, Figure 5C) and MaxN and snorkel densities ($r^2 = 0.62$, $p < 0.001$, Figure 5D).

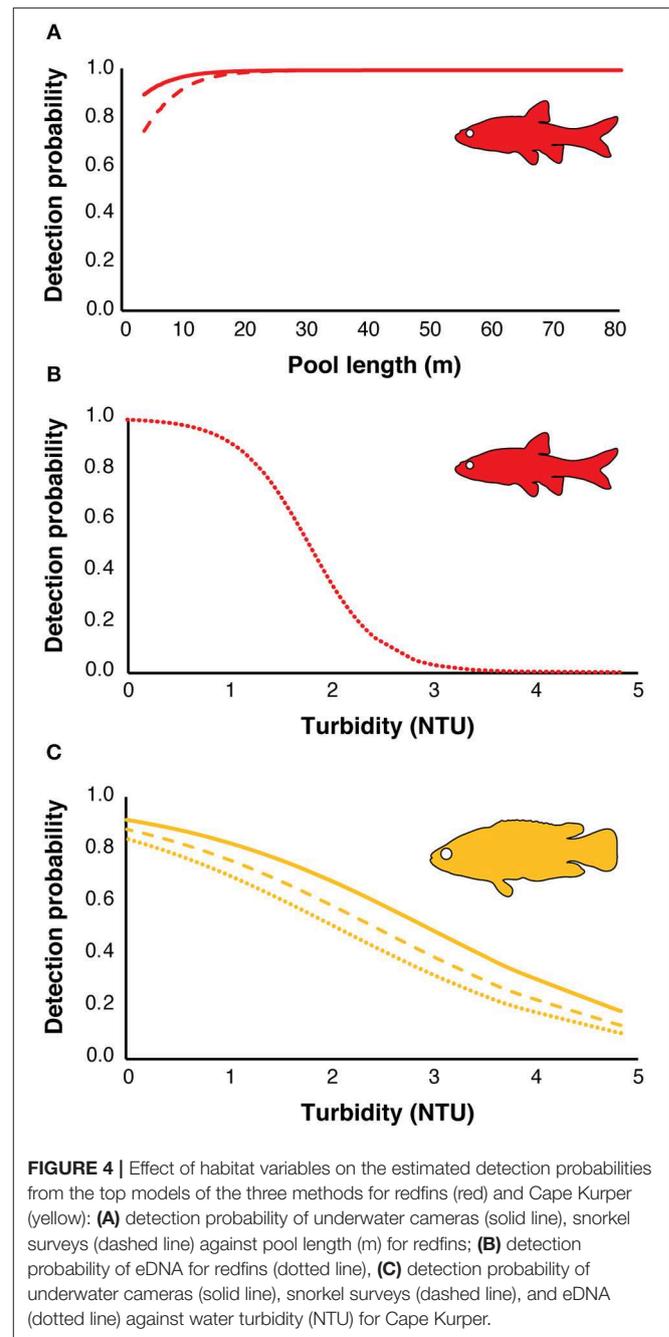


FIGURE 4 | Effect of habitat variables on the estimated detection probabilities from the top models of the three methods for redfins (red) and Cape Kurper (yellow): **(A)** detection probability of underwater cameras (solid line), snorkel surveys (dashed line) against pool length (m) for redfins; **(B)** detection probability of eDNA for redfins (dotted line), **(C)** detection probability of underwater cameras (solid line), snorkel surveys (dashed line), and eDNA (dotted line) against water turbidity (NTU) for Cape Kurper.

Fish Abundance and Pool Morphology Differ Between Tributaries

For both fishes, abundance collected by snorkel survey was significantly higher in the Blindekloof than the other three tributaries (Kruskal-Wallis test, Dunn's *post-hoc* test, $p < 0.05$, Figures 6A,B), but that relationship was lost when the count data were converted to densities (m^{-2} , Figures 6C,D). The Blindekloof also had the longest, widest, and deepest pools, followed by the Bos, and no significant differences were found between the Fernkloof and Waterkloof (Kruskal-Wallis test, Dunn's *post-hoc* test, $p < 0.05$; Figure 7).

TABLE 3 | Summary of the model selection using AIC for the occupancy and detection probability of Cape Kuper using underwater cameras, snorkel surveys, and combined eDNA.

Model $\psi(\text{width}), \theta(\cdot)$	AICc	Δi	w_i	K	-2Log(L)
$p(m + \text{turb})$	157.18	0.00	0.24	7	141.34
$p(\text{camera\&snorkel} + \text{turb}, \text{eDNA}(\text{avg width}))$	157.96	0.78	0.16	8	139.56
$p(\text{camera\&snorkel} + \text{turb}, \text{eDNA}(\text{avg depth}))$	158.57	1.39	0.12	8	140.17
$p(\text{camera\&snorkel} + \text{turb}, \text{eDNA}(\cdot))$	158.91	1.73	0.10	7	143.07
$p(\text{camera\&snorkel} + \text{turb}, \text{eDNA}(\text{turb}))$	159.55	2.37	0.07	8	141.15

Top five models are presented.

m and θ , method-specific detection probability; avg, average; +, covariate effects both methods similarly.

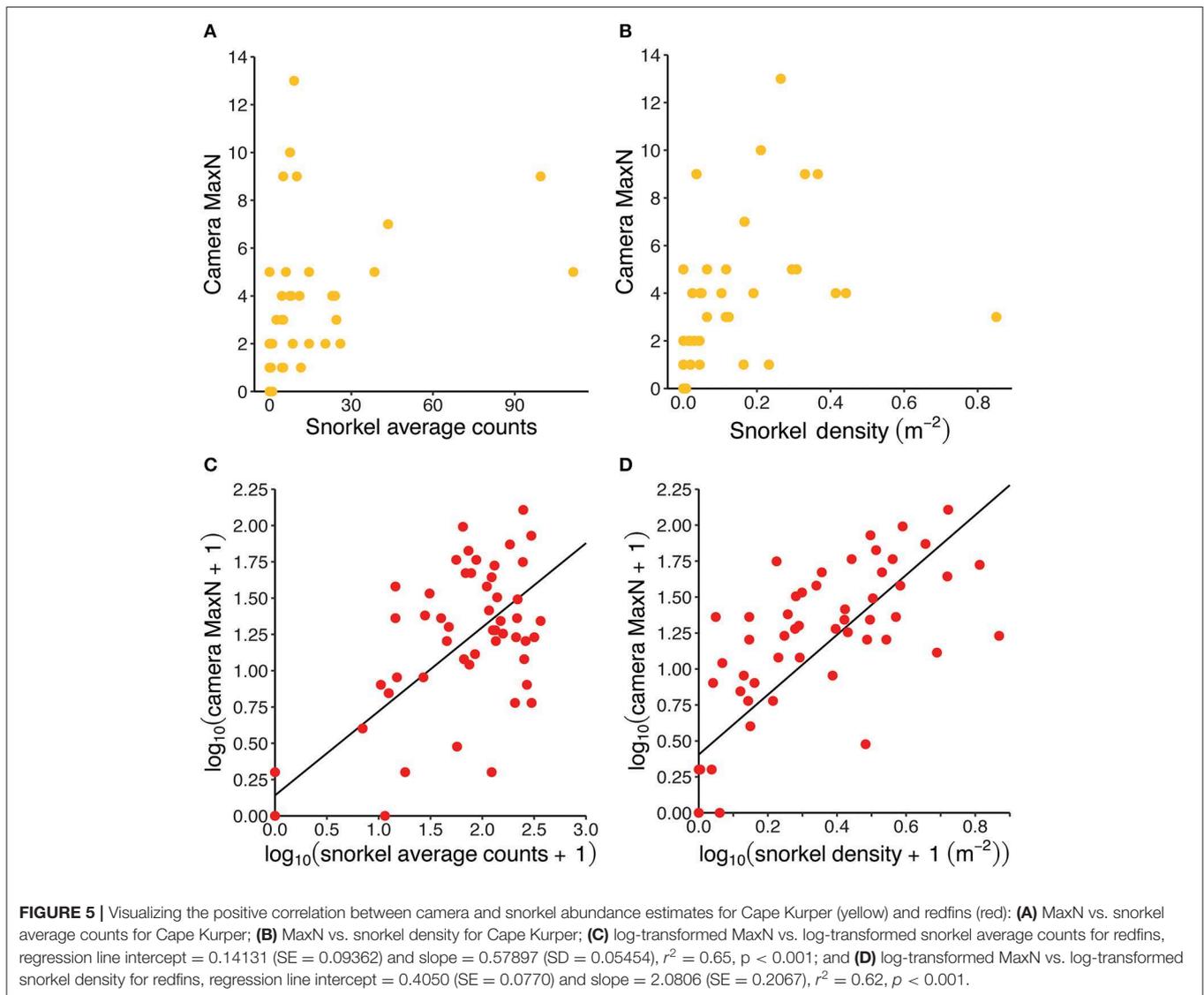
DISCUSSION

The model-averaged detection probabilities of the three non-invasive methods were higher for redfins than Cape Kurper and were affected by different habitat covariates. For both fishes, eDNA had the lowest detection probability and was negatively affected by turbidity. However, the model-averaged detection probability for Cape Kurper was similar between snorkel surveys and eDNA. Overall, underwater cameras and snorkel surveys had similar capabilities in detecting the fish community. For the two IUCN-listed fishes, underwater cameras had the highest detection probability compared to snorkel surveys and eDNA. For redfins, the detection probabilities of the visual observation methods were positively influenced by pool length. For Cape Kurper the detection probabilities of the visual observation methods were negatively influenced by turbidity. There were strong correlations between MaxN and abundance estimates, and MaxN and density. However, the linear relationship showed low goodness of fit, making it difficult to directly convert MaxN to absolute abundances.

The detection probabilities of redfins and Cape Kurper using eDNA were the lowest among the three gear types, but it detected both species in all four tributaries. While slightly lower than the visual observation methods, detection probability is within the range of other eDNA studies of rare freshwater species in lotic habitats (e.g., Schmidt et al., 2013; de Souza et al., 2016; Lopes et al., 2017; Bylemans et al., 2019; Sutter and Kinziger, 2019), but was highly dependent upon sampling strategy including volume of water filtered (Wilcox et al., 2016; Cantera et al., 2019; Hunter et al., 2019), number of PCR technical replication effort (Piggott, 2016), size, material, and pore size of the filter (Majaneva et al., 2018), the seasonal hydrology of the system, and rarity of the target (Buxton et al., 2017). Some of these factors, such as rarity, cannot be controlled and are specific to the species under investigation. For example, Wilcox et al. (2016) found that qPCR-based methods could detect a population density of a single fish within a 1 km stretch of river with probability of 0.18, which increased to >0.99 for three fish within a 100 m stretch. Other factors can be mitigated by increasing sampling

effort but, as a result, also increase the cost associated with the study. In our study, based on estimates for individual pools and despite an overall lower value of detection probability, the sensitivity of eDNA was such that the scale of detection of both the Cape Kurper and the redfins was effective for detection at the watershed level. For these reasons, eDNA and visual methods may be better suited for detecting species at different spatial scales. The high sensitivity and efficiency of eDNA makes it particularly useful for large rivers. At this scale, eDNA methods outperform conventional sampling methods (Pont et al., 2018), including visual surveys (Lopes et al., 2017; Nakagawa et al., 2018). In contrast, visual observation methods may outperform eDNA when the occupancy of specific pools is of interest. False negatives are more likely for an individual pool, especially if the hydrochemical properties of the pool inhibit the detection of eDNA (e.g., high turbidity; Jane et al., 2015). False positives may be even more problematic at this spatial scale, as eDNA can be deposited in a pool from a source many kilometers upstream (Jane et al., 2015; Balasingham et al., 2017; Pont et al., 2018). The effectiveness of eDNA for characterizing species assemblages across the longitudinal stretch of a river has not been extensively investigated and, because of the many factors contributing to the preservation of eDNA in the water column, may be highly variable across systems (Nakagawa et al., 2018; Pont et al., 2018). In addition, most studies have focused on lentic or marine environments when comparing visual and eDNA methodologies (Yamamoto et al., 2016; Doble et al., 2019). Thus, currently, visual methods may be most appropriate for studies aimed at identifying pools within a river system for rehabilitation or protection, whereas eDNA may be most effective for large scale-studies.

The habitat covariate that had the greatest effect on eDNA detection for both redfins and Cape Kurper is turbidity. Turbidity, a proxy of organic solids containing PCR inhibitors, such as tannic and humic acids, has severe negative effects on the detection of species using eDNA, and increases susceptibility of the survey to false negatives (Harper et al., 2019). PCR inhibitors may also have unpredictable effects on increasing the volume of water sampled (Cantera et al., 2019). The impact of inhibitory substances can be alleviated by diluting samples prior to amplification; however, this practice will also decrease concentrations of target DNA. For rare species, this may dilute target DNA beyond the LOD (Sutter and Kinziger, 2019; Skinner et al., 2020). Methods for removing PCR inhibitors have also been developed (Hu et al., 2015). The detection probability of the Cape Kurper, the rarer species in these pools, is lower for all gear types, which may reflect its lower abundance. However, detection probability with eDNA is comparable for both species, and for the Cape Kurper is comparable to visual methods. This highlights the high sensitivity of eDNA studies, even for rare cryptic species such as the Cape Kurper, while more active and conspicuous species like the redfins might be easier detected with visual methods. Behavioral factors may also impact detection probability using eDNA. For example, differences in shedding rates (Klymus et al., 2015), and activity patterns might also have an impact on the eDNA suspended in the water column (de Souza et al., 2016). The Cape Kurper predominantly swims in short



rapid movements and is more associated with the benthos than the redfins (Kadye et al., 2016). This exemplifies the importance of considering the specific biology of species when designing sampling protocols. For example, Cape Kurper may be more easily detected from sediment samples or water taken from deeper in the water column. Sampling methods combining water samples from different depths (Doble et al., 2019) and sediment samples may improve the detection of specific species, including the Common Carp (Turner et al., 2015), an invasive species found in the same systems in eastern South Africa.

Overall, the sensitivity of our eDNA assay could be improved by increasing the volume of water sampled and the number of biological and technical replicates, and by removing or mitigating the effect of inhibitors; however, this would lead to additional technical challenges associated with transporting or processing water samples, especially for remote localities. In this study, including the detections and non-detections of each eDNA

replicate of each pool, instead of using the combined-replicate eDNA detection and non-detections, into the occupancy models reduced the eDNA detection probability by about 20%, revealing that there is a lot of variation in detection across replicates taken from a pool. Increasing the number of physical replicates and the total volume of water sampled should improve detection probability of both species, assuming inhibition of the PCR reaction can be overcome (Cantera et al., 2019). In addition, for these remote locations, filtering on site is also more appropriate to limit degradation of eDNA and reduce effort required to transport samples. Recent methodological advances have made onsite eDNA extraction and PCR-based detection easier, but are not inexpensive (Thomas et al., 2018). Costs associated with acquiring or transporting specialized equipment and the increased personnel hours required to increase sampling effort and onsite processing may be disproportionately high in remote areas.

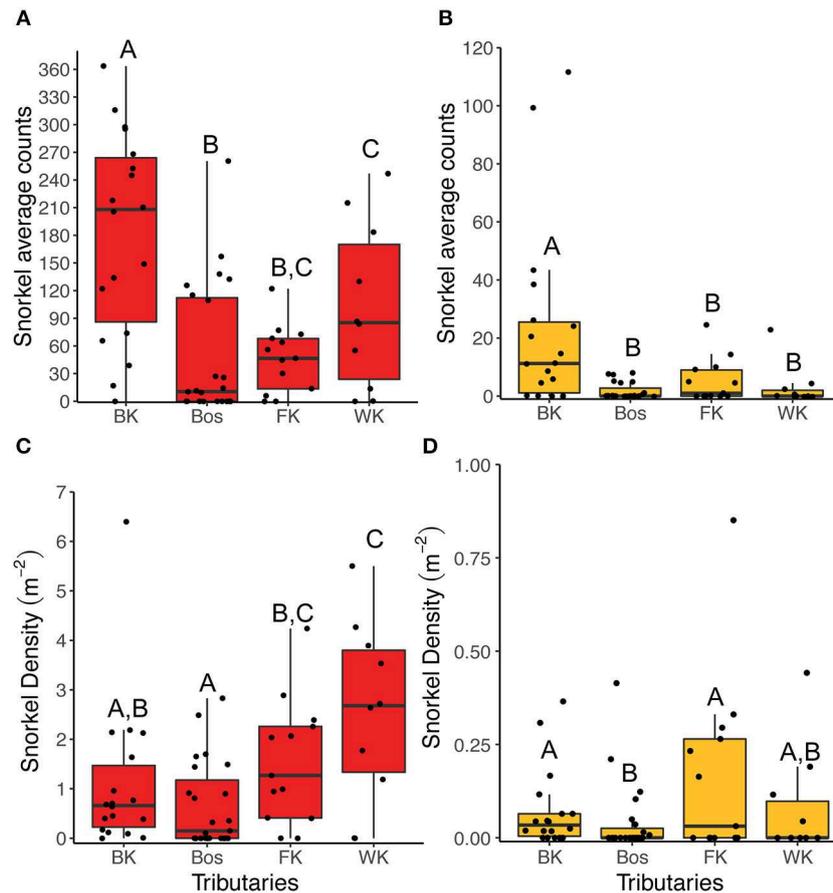
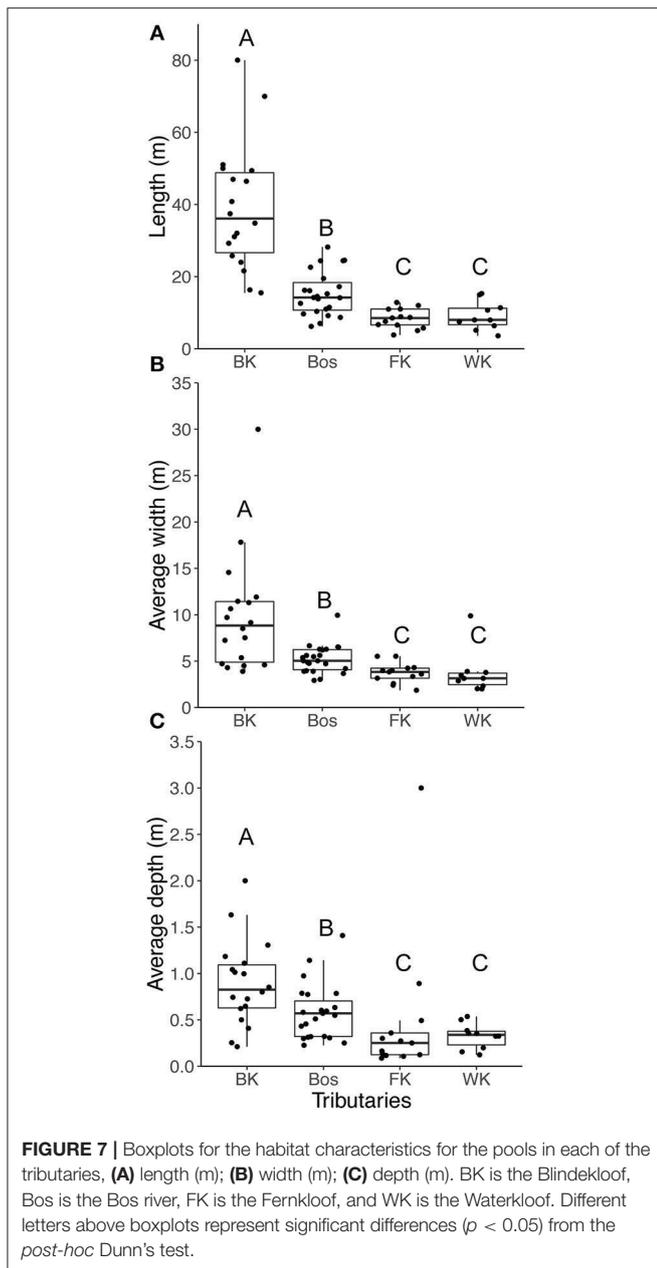


FIGURE 6 | Boxplots of the snorkel abundance data collected for redfins (red) and Cape Kurper (yellow) at each tributary. The snorkel average counts for **(A)** redfins, and **(B)** Cape Kurper. The snorkel densities (m⁻²) for **(C)** redfins, and **(D)** Cape Kurper. BK is the Blindekloof, Bos is the Bos river, FK is the Fernkloof, and WK is the Waterkloof. Different letters above boxplots represent significant differences ($p < 0.05$) from the *post-hoc* Dunn's test.

The most significant costs associated with processing eDNA samples are associated with the facilities and instruments required for the extraction and PCR (Hinlo et al., 2017). Because of the high risk of contamination, it is essential to maintain two functional lab spaces, one “clean lab” where samples can be extracted and processed separate from the lab where the PCR reactions are undertaken (Goldberg et al., 2016). Therefore, the cost associated with these studies is largely dependent on the accessibility of the required facilities and is many orders of magnitude more expensive than visual methods. These costs shrink as the size of the study increases due to economies of scale and the high-throughput capacity of qPCR assays and next-generation sequencing (Smart et al., 2016). Nevertheless, even if the appropriate facilities for processing eDNA samples are available, methods need to be optimized for the target species, ensuring a minimum acceptable standard for deployment (Klymus et al., 2020), analogous to the MIQE standards in clinical PCR applications (Bustin et al., 2009). Once optimized, these approaches are widely applicable across broad geographic scales, taking into account environmental factors that may influence their efficacy (Cantera et al.,

2019). The standardized nature of these studies has made it easier to study wide-spread invasive (Amberg et al., 2015) and commercially valuable fishes (Thomsen et al., 2016), and make detection studies more reproducible (Mauvisseau et al., 2019). In contrast, investigations of new species, typical in understudied localities, require careful optimization of sampling methods, laboratory protocols, and development of species-specific primers. This process can incur unexpected costs as the success of sampling approaches, primers, and protocols can vary unpredictably.

Generally, underwater cameras detected all the same fish species captured by snorkel surveys, although there were slight differences. Cameras detected Goldie Barb and River Goby fewer times than by snorkeling, although the differences were not significant. Nevertheless, differences in detection percentages revealed that underwater cameras did not capture Goldie Barb and River Goby as effectively as snorkel surveys, which may be due to the ecology and morphological characteristics of the fishes. Both the Goldie Barb and River Goby have translucent, light-brown slender bodies (Skelton, 2001) that can be difficult to see and identify in video images. Further, River Goby is a cryptic



bottom-dwelling fish that tends to remain among cover (Skelton, 2001), making detection using a stationary camera more difficult as cameras rely on animal movement to record encounters (Rowcliffe et al., 2008). The tripod-mounted camera may not be the most effective set up for benthic fishes; a camera pointing toward the sediment mounted on a tow seems to effectively detect and quantify Round Goby, a benthic fish (Schaner et al., 2009; Taraborelli et al., 2009). Although underwater cameras were not as effective as snorkeling in detecting Goldie Barb and River Goby, they detected African Longfin Eel and African Sharptooth Catfish, which snorkel surveys did not. Both of these fishes tend to be more active at night and hide among rocks during the day (Kadye and Booth, 2013; Ellender et al., 2018);

therefore, opportunistic sightings of these fishes leaving their shelter when not disturbed (e.g., by a snorkeler) were captured with underwater cameras. To capture the whole community, both diurnal and nocturnal fishing, using visual observation methods, night-time sampling would be required. Ellender et al. (2018) performed night snorkeling with a strong dive flashlight to observe the behavior of native eels, and other studies have used infrared cameras or light attachments on cameras for nocturnal-fish studies (Bassett and Montgomery, 2011; Dirnwoeber et al., 2012; Harvey et al., 2012). Night sampling would require additional personnel hours and extra safety precautions. Alternatively, eDNA could be used to detect the entire fish community with water samples taken from a pool without the necessity to sample at night.

For the endangered redfins, differences were observed in detection probabilities between visual observation methods. The model-averaged estimates revealed that underwater cameras had a higher detection probability and lower variation than snorkel surveys and eDNA. The detection probabilities for underwater cameras and snorkel surveys were above 0.90, suggesting that both methods are very effective at detecting redfins, although these detection probabilities decrease with decreasing pool length. The decreasing detection probability of both visual methods with decreasing pool length for redfins is contrary to what we had hypothesized. Generally, as the spatial scale of an area increased, it became less likely that an animal would be encountered; however, if the number of individuals also increased, then the animal was more likely to be encountered (Rowcliffe et al., 2008). Redfins abundance was highest in the Blindekloof, which also had the longest pools, but density was higher in smaller river pools. If the fish were diluted across the length of the pool as indicated by the density estimation, then detection probability would decrease, as we hypothesized, not increase as the models revealed. However, Redfin tend to form large shoals of over 100 individuals (Skelton, 2001; Ellender et al., 2018), which are very conspicuous and readily detectable by the underwater camera and snorkeler, especially as it is a curious fish that is attracted to the snorkeler (RAC, personal observation). Ellender et al. (2012) found that these two fish species were attracted to the cameras <5% of the time, noting that the behavior does not bias the camera detection data. Therefore, detection probabilities of the visual observation methods are likely being driven by the large schools of redfins in the long pools of the Blindekloof. A study by Castañeda et al. (2020) revealed that underwater cameras had a high detection probability in detecting an endangered minnow that mainly used the water column of a pool, similar to redfins. On the other hand, Cape Kurper does not aggregate in large shoals; thus, its density likely reflects its actual distribution across the pools and the detection probability of both visual observation methods is hypothesized to decrease with increasing spatial scale.

The detection probabilities of the Cape Kurper were lower than redfins for all gear types. The detection probability of underwater cameras for Cape Kurper was 23% lower than that of redfins, while the snorkel survey detection probability was 28% lower. The habitat covariate that drives the detection probabilities for the visual observation methods of the Cape Kurper also

differs from that observed for the redfins. Turbidity, not a spatial covariate as with redfins, had the strongest negative effect on the detection probabilities on the two visual methods; as water clarity decreases so does detection probability of the two methods that solely rely on vision to capture fishes. Cape Kurper is more cryptic relative to the large shoals of redfins and tends to hide in structural habitat (Ellender et al., 2012), thus it would be difficult to detect in less clear water. Higher water turbidity also decrease the detection probability of underwater cameras and backpack electrofishing, two visual methods that have been applied for monitoring an endangered minnow in temperate streams in Canada (Castañeda et al., 2020). A study conducted by Ellender et al. (2012) in the Swartkops watershed confirmed that there were no significant differences between the detection rates of underwater cameras and backpack electrofishing for either redfins and Cape Kurper, concluding that underwater cameras are a good non-invasive alternative method.

Estimating abundance and density is required to attain important information on the population ecology of these fishes; hence, direct comparison between these estimates is necessary. There was a strong correlation between the camera and snorkel data, MaxN increased with both increasing snorkel counts and density. For redfins, log-transformation successfully normalized the data, thus linear regressions could be run. The linear regressions revealed that there was a direct relationship between MaxN and snorkel counts and density; however, the goodness of fit values were low. The goodness of fit values suggests that only about 60% of the variance of MaxN could be explained by snorkel counts and density. For Cape Kurper, because the data were not normally distributed even with data-transformation techniques, a linear regression could not be run without violating important assumptions. Therefore, no true pattern could be discerned for direct conversion between MaxN and the snorkel data. Furthermore, cameras are static and do not have a spatial reference, unless used in stereo or the distance to a fixed known point is calibrated (Harvey et al., 2002; Struthers et al., 2015) to appropriately convert MaxN to density, resulting in measurement of relative, not absolute, abundance as with snorkeling that covers the whole pool. At most, researchers using underwater cameras can conclude that a higher MaxN recording results in a higher relative abundance of fish, as the positive correlations revealed in this study and the lack of fit between MaxN and density. Generally, MaxN often underestimates abundance while snorkel surveys can be biased based on the behavior of the fishes (Harvey et al., 2002; Campbell et al., 2015). Hence, if absolute abundances are required, then snorkel surveys are better suited than underwater cameras, as the latter could only provide relative abundance estimates, unless multiple cameras are deployed to cover the entire pool.

There are strengths and weaknesses for each method, and researchers must prioritize their conservation needs and resource availability before deciding on which method to use for sampling in remote locations. In clear headwater environments, visual observation methods were better at detecting IUCN-listed fishes than eDNA. Visual observation methods performed best in clear waters as increasing water turbidity decreased the field of view

of the snorkelers and cameras; therefore, other non-invasive methods are needed when turbidity is high. However, given that eDNA was similarly negatively influenced by turbidity, it is unsuitable to replace visual observation methods in streams with higher turbidity. For all three methods, depending on the distance between pools, many sites can be sampled in a single day; however, it could take many hours of trained-personnel time to score video data (~35 min per video, RAC personal observation) and process eDNA samples. Furthermore, electricity, which may not be available in remote field sites, is needed to charge camera batteries, upload video footage to hard drives, and filter eDNA if not using a hand pump. In this study, we used only one camera per pool; however, depending on the research needs and environmental factors, different approaches can be used. For example, the use of two stereo-cameras mounted at a fixed distance, calibrated with a scale bar, can accurately measure the size of fishes for population-structure analysis (Harvey et al., 2002; Harasti et al., 2014), or multiple cameras can be used at a site to increase detection of rare fishes (Castañeda et al., 2020). An additional resource necessary for eDNA at the base camp is a dedicated sterile space for eDNA filtration, which may not be readily available in many remote areas. Personnel trained on sterile filtration technique would be necessary to process the eDNA on site. Nevertheless, eDNA could detect full species community (diurnal and nocturnal fishes) with one visit, unlike the visual observation methods that would require night-time sampling. On the other hand, snorkel surveys require trained personnel in the field to reliably count and identify species in the streams, but the data are immediately available for analysis. However, fish identifications and counts using snorkel surveys are not verifiable unless visually recorded (e.g., GoPro on mask), and information regarding behavior can be difficult to collect without disturbing the fish, as they may be attracted to or repelled by the snorkeler if the snorkeler moves too quickly (Thurrow et al., 2012). Specialized fish identification training would be required for field personnel. Snorkel surveys can provide density data while underwater cameras can only reliably provide relative abundance. Generally, eDNA requires the most specialized space and equipment but has the greatest potential for high-throughput assays, once optimized for an ecological community or locality. Underwater cameras are a powerful tool capable of capturing and storing unique information on the behavior and habitat use of fishes but cost more and take more time to complete than snorkel surveys, especially if multiple cameras are needed (Friesen and Chivers, 2006; Domenici et al., 2014; Struthers et al., 2015). The equipment needed to collect the field data for the three methods can easily be transported through rugged terrain with the main limiting factor for the three methods being accessibility to resources in the lab and field.

Researchers are encouraged to use at least one of these non-invasive methods when sampling rare freshwater fishes as they are reliable tools for collecting information on fish ecology in remote areas with minimal negative effects on the populations themselves. Climate change is predicted to increase water temperatures and alter water discharge, which may have negative impacts of the growth and survival of freshwater fishes (Bassar et al., 2016; Reid et al., 2019). Fishes

listed on the IUCN Red List are already threatened by other stressors such as habitat degradation and invasive species. The additional threat of climate change may compound or work synergistically with existing threats and exacerbate stress on already vulnerable fishes (Rahel and Olden, 2008; Comte and Olden, 2017; Reid et al., 2019). Using non-invasive sampling methods to reduce unintentional harm during monitoring will become more important as multiple threats continue to impact fish populations and communities. Continual monitoring of vulnerable fish populations is important to understand occupancy and metapopulation dynamics across time and space and to make appropriate inferences on conservation management needs; these non-invasive methods can facilitate long-term monitoring studies without jeopardizing populations. Therefore, these three methods will become of even greater importance as climate change and other stressors continue to negatively impact unique fish communities throughout freshwater ecosystems (Darwall et al., 2018; Reid et al., 2019).

DATA AVAILABILITY STATEMENT

The datasets generated for this study are available on request to the corresponding author.

ETHICS STATEMENT

The animal study was reviewed and approved by SAIAB Animal Ethics Committee University of Toronto Scarborough Local Animal Care Committee.

AUTHOR CONTRIBUTIONS

RC, SC, BE, and OW conceived the project. RC, SC, and BE designed the field protocol and collected the field data. AV and SC completed the eDNA extractions. AV designed and conducted the qPCR protocol and wrote the methods. RC scored the video

recordings. RC analyzed and interpreted the data, and wrote the manuscript with assistance from AV, SC, NM, and OW. DH, HM, NM, and OW financially supported, approved, and provided critical advice on the project. All co-authors have revised the manuscript and gave final approval for publication.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fenvs.2020.00029/full#supplementary-material>

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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