Chapter Two: Study Areas with General Materials and Methods

2 Study areas with general materials and methods

2.1 Introduction to study areas

To reach the aims and objectives for this study, one lentic and one lotic system within the Vaal catchment had to be selected. The lentic component of the study involved a manmade lake or reservoir, suitable for this radio telemetry study. Boskop Dam, with GPS coordinates 26°33'31.17" (S), 27 °07'09.29" (E), was selected as the most representative (various habitats, size, location, fish species, accessibility) site for this radio telemetry study. For the lotic component of the study a representative reach of the Vaal River flowing adjacent to Wag 'n Bietjie Eco Farm, with GPS coordinates 26°09'06.69" (S), 27°25'41.54" (E), was selected (Figure 3).

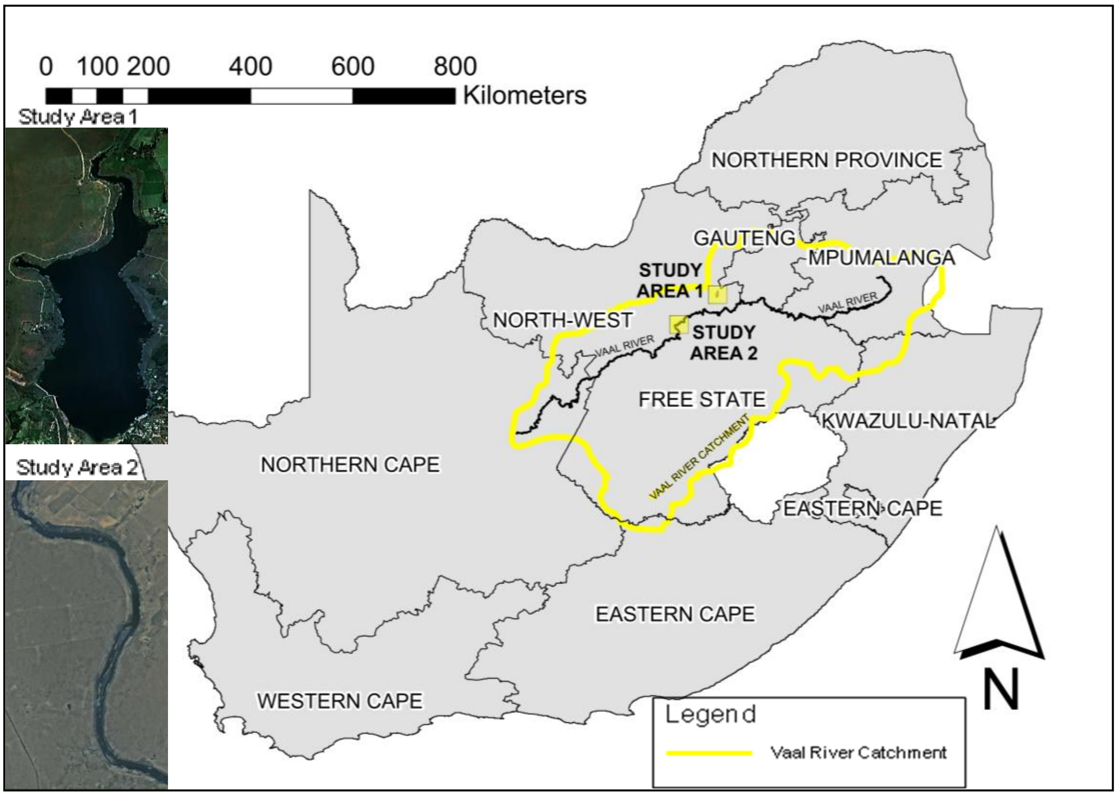

Figure 3: Map of the two study areas within the Vaal River catchment, South Africa

Boskop Dam

Boskop manmade lake also known as Boskop Dam is situated 15 km north of Potchefstroom (Figure 4) in the Dr. Kenneth Kaunda District Municipality in the North West Province (Van Aardt and Erdmann, 2004). The dam is part of the Mooi River water scheme and is currently the largest reservoir built on the Mooi River (Koch, 1975). Apart from Boskop Dam, two other manmade lakes can be found on the Mooi River including Kerkskraal and Lakeside Dam (also known as Potchefstroom Dam). The Mooi River rises in the north near Koster and then flows south into Kerkskraal Dam which feeds Boskop Dam. Boskop Dam stabilises the flow of the Mooi River and two concrete canals convey water from the Boskop Dam to a large irrigation area. The Mooi River then flows about 20 km in a southerly direction and reaches Potchefstroom Dam. From there the Mooi River enters the Vaal River at GPS 26°52'27.31" (S), 26°57'06.33" (E) to form an important tributary (Koch, 1975). Boskop Dam was completed in 1959 with a total dam-wall length of 1 320 m (DWAF, 2009). This reservoir can hold a maximum capacity of $21x10^6$ $m^3$with an annual outlet capacity of $5.6x10^6$ m^3 (DWAF, 2010). The littoral zone around the lake is mostly covered with an aquatic weed *Potamogeton pectinatus* (Koch and Schoonbee, 1975). This weed invaded 50% of the total surface area of the lake in 1975, and this percentage has remained more or less constant (Koch and Schoonbee, 1975).

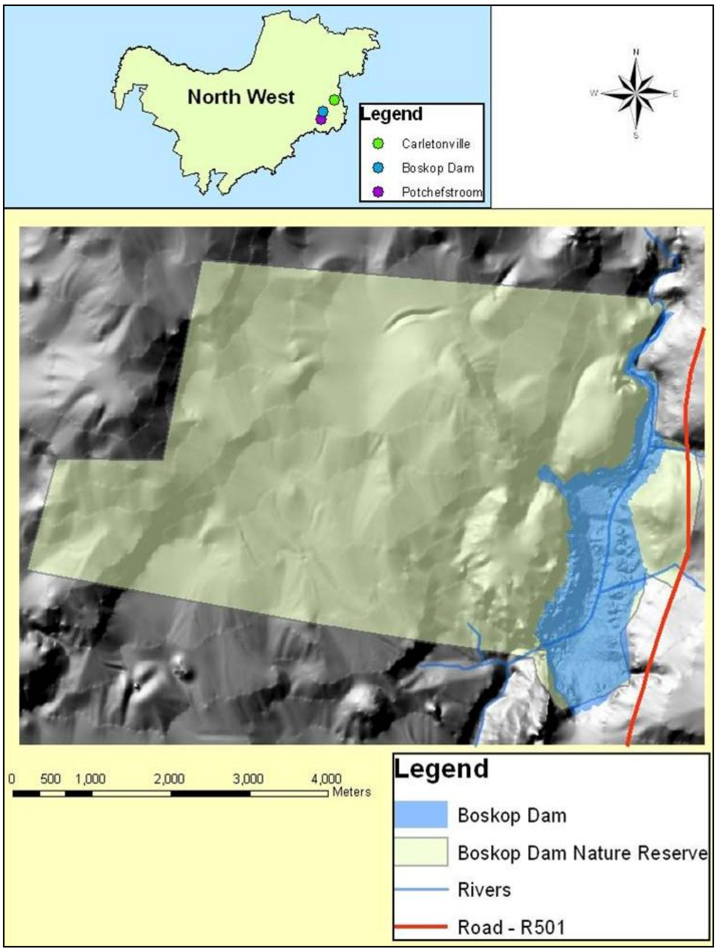

Figure 4: Map of study area 1: Boskop Dam situated 15 km north of Potchefstroom within Boskop Dam Nature Reserve in the North West Province, South Africa

Due to the clarity of the water in Boskop Dam, sufficient sunlight penetrates the water and allows plants to grow in depths of up to 6 m (Brand, 1975). In addition to weeds and plants, Boskop Dam has a large diversity of habitats available. These habitats include aquatic vegetation that can be 200 m wide in some areas (Figure 5A-C), boulders (Figure 5D), shallow gravel beds (Figure 5E-F) and deep habitats with reeds (Figure 5G-H) surrounding the entire edge of the reservoir (Skelton, 2001). Boskop Dam is situated in a summer rainfall region and receives an average annual rainfall of 649 mm and has an average summer temperature range of 22°C to 34°C with a winter temperature range of 2°C to 20°C. Average water temperatures usually range between 11°C in winter and 26°C in summer (Koch and Schoonbee, 1975). This lentic system is situated inside Boskop Dam Nature Reserve, a sanctuary extending over an area of 3 000 ha (Van As and Combrinck, 1979). Access to Boskop Dam is mainly controlled by personnel of the North West Parks Board, but private land owners on its eastern bank and the Department of Water Affairs on its southern bank have permanent access to the system.

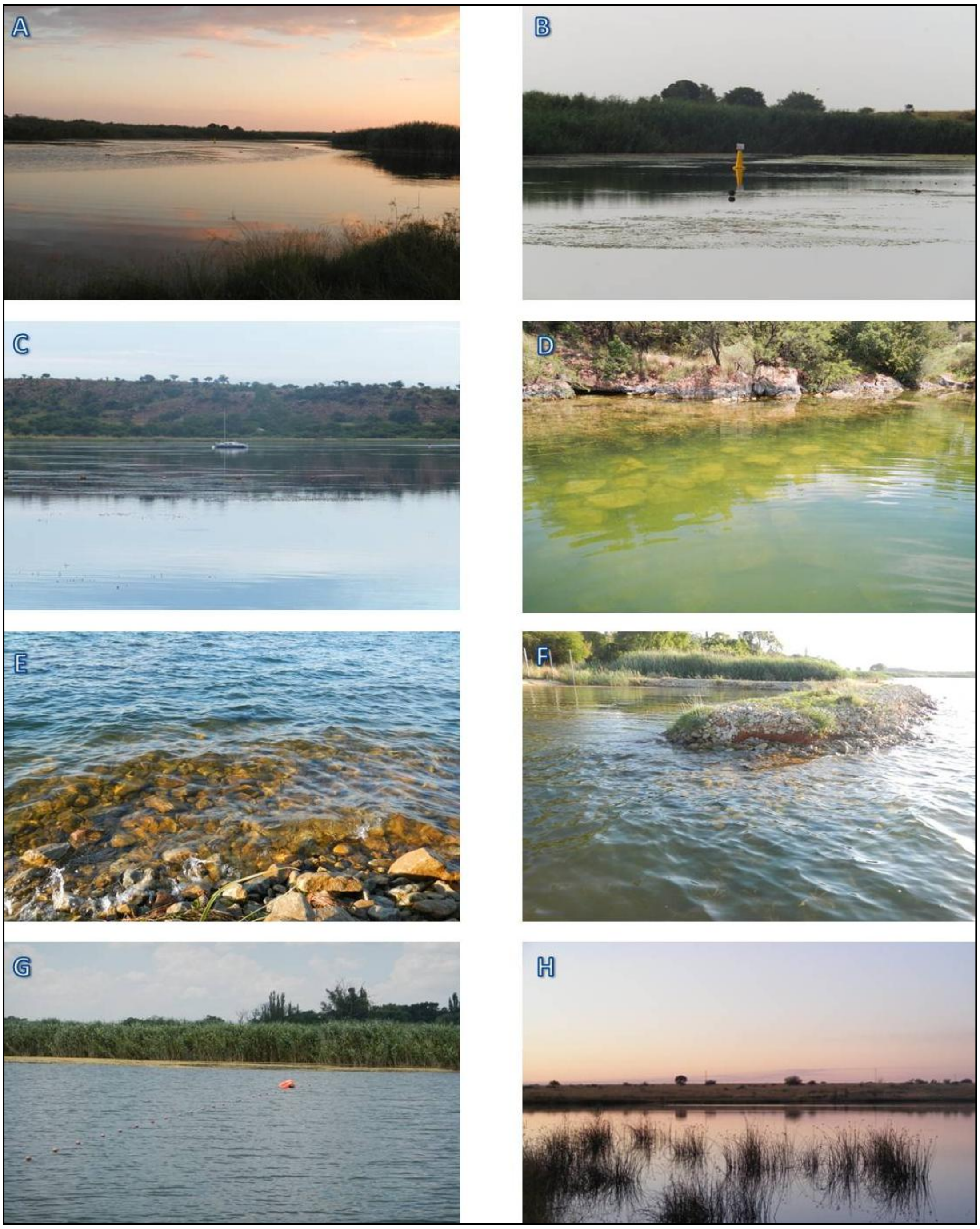

Figure 5: Habitats in Boskop Dam include aquatic vegetation (A-C); boulders (D); shallow gravel beds (E-F); and deep water with reeds surrounding entire study area (G-H)

Vaal River

The lotic component of the study is a reach of the Vaal River situated downstream of the Orkney weir and about 125 km upstream of Bloemhof Dam, in the Middle Vaal Water Management Area (WMA) (Figure 6). The reach is 10 km in length and situated in a wilderness area controlled by Orange-Vaal River Yellowfish Conservation and Management Association (OVRYCMA) members. As a result, the entire area was closed to other water-related recreational activity users throughout the experiment, thereby minimising disturbance to yellowfish monitored in the study.

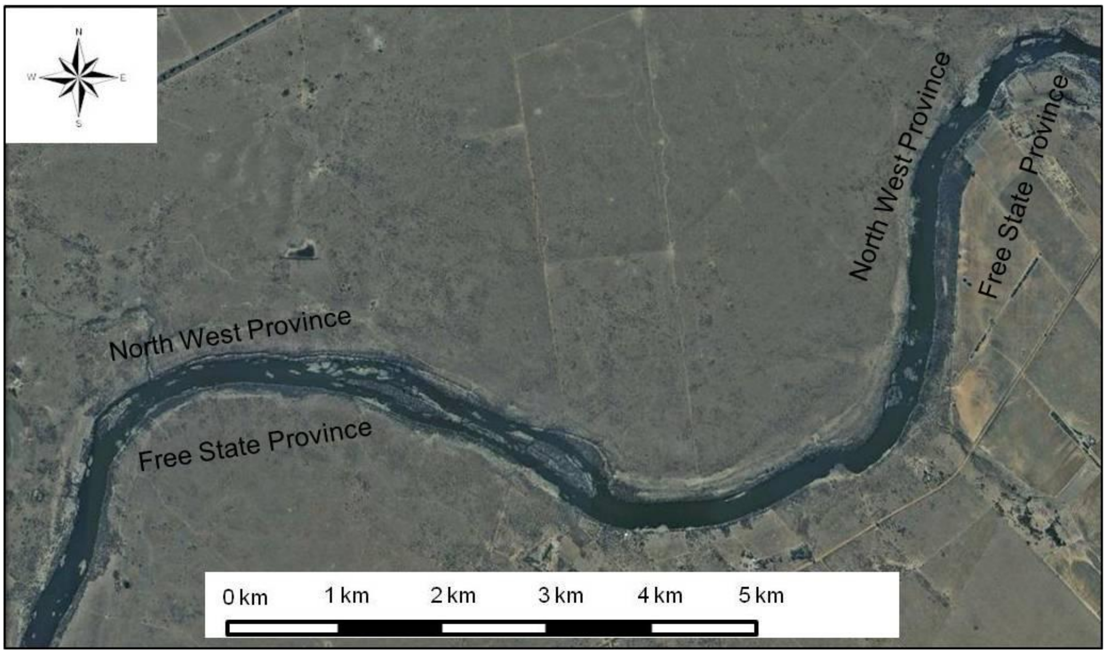

Figure 6: Map of study area 2, a reach of the Vaal River flowing through Wag ‘n Bietjie Eco Farm, on the border between North West Province and Free State Province, South Africa

The area contained a large diversity of habitat types, including deep pools (Figure 7A), undercut banks with submerged roots and trees (Figure 7B), fast rapids, riffles with reeds and vegetation (Figure 7C), sand, gravel beds with boulders (Figure 7D-E) and aquatic vegetation (Figure 7F). This lotic system is situated in a summer rainfall region and receives an annual rainfall of 500 mm to 600 mm (Støwer, 2013). In addition, large parts of the Vaal River upstream have been transformed in many ways; these include quality of the water, quantity alterations, timing and duration of flows, habitat modifications and impacts associated with alien invasive species (Davies and Day, 1998). No barriers or point-source pollution impacts that might influence the natural movement of yellowfish were present in the study area (Davies and Day, 1998; Van Wyk, 2001; Nel *et al*., 2007).

Figure 7: The Vaal River study area has a large diversity of habitat types, including deep pools (A); undercut banks with submerged roots and trees (B); fast rapids, riffles with reeds and vegetation (C); sand, gravel beds with boulders (D-E); and aquatic vegetation (F)

2.2 Suitability of the study areas

Boskop Dam

Boskop Dam was selected as the most representative site for this radio telemetry study; however, very little information on fish species occurring in this reservoir exists. Information collected on fish species in Boskop Dam included the following case study: A Fish Mark-Recapture Study, Boskop Dam, Western Transvaal by Koch and Schoonbee (1975). Their study resulted in 35 253 fishes being collected. Of these 35 253 fishes: 85.71% were *Labeo capensis* (A. Smith, 1841), 9.28% *Labeo umbratus* (A. Smith, 1841), 0.31% *Cyprinus carpio* Linnaeus, 1758, 0.15% *Clarias gariepinus* (Burchell, 1822), 0.05% *Micropterus dolomieu* (Lacepéde, 1802), 3.63% *Tilapia sparmanii* (A. Smith, 1840), and 0.87% were *L. aeneus* (Koch and Schoonbee, 1975). This study concluded that there was a healthy yellowfish population in Boskop Dam, but it was carried out 37 years ago. It was therefore necessary to carry out fish suitability assessment of Boskop Dam to ensure that there is a healthy yellowfish population available, that can be used for this radio telemetry study. Although the suitability assessment was aimed at identifying healthy yellowfish populations, information on all species occurring in Boskop Dam would be collected (Table 5).

Table 5: Various fish species that could occur in Boskop Dam, including order, family, taxon and common names, alien fish species are identified with an * in the table (Skelton, 2001)

Order	Family	Taxon	Common name
Cypriniformes	Cyprinidae	*Barbus anoplus*	Chubby-head barb
Cypriniformes	Cyprinidae	*Barbus pallidus*	Goldie barb
Cypriniformes	Cyprinidae	*Barbus paludinosus*	Straight-fin barb
Cypriniformes	Cyprinidae	*Barbus trimaculatus*	Three spot barb
Cypriniformes	Cyprinidae	*Ctenopharyngodon idella*	Grass carp*
Cypriniformes	Cyprinidae	*Cyprinus carpio*	Common carp*
Cypriniformes	Cyprinidae	*Labeo capensis*	Mudfish
Cypriniformes	Cyprinidae	*Labeo umbratus*	Moggel
Cypriniformes	Cyprinidae	*Labeobarbus aeneus*	Smallmouth yellowfish
Cypriniformes	Cyprinidae	*Labeobarbus kimberleyensis*	Largemouth yellowfish
Perciformes	Centrarchidae	*Micropterus salmoides*	Largemouth bass*
Perciformes	Centrarchidae	*Micropterus dolomieu*	Smallmouth bass*
Perciformes	Cichlidae	*Pseudocrenilabrus philander*	Southern mouthbrooder
Perciformes	Cichlidae	*Tilapia sparmanii*	Banded tilapia
Siluriformes	Austroglanididae	*Austroglanis sclateri*	Rock catfish
Siluriformes	Clariidae	*Clarias gariepinus*	Barbel

To assess the availability of yellowfish species in Boskop Dam, different methods were used, including gill nets (Figure 8A), fyke net traps (Figure 8B), seine nets (Figure 8C), electro-fishing (Figure 8D), angling (Figure 8E) and visual observations (Figure 8F).

Figure 8: Methods used to assess the suitability of Boskop Dam included gill nets (A); fyke net traps (B); seine nets (C); electro-fishing (D); angling (E); and visual observations (F-H)

Vaal River

The Vaal River study area has been used to carry out numerous research studies throughout the past seven years. In addition, this area is a well-known angling destination in South Africa. It was therefore not necessary to carry out a suitability assessment of the area as numerous suitable yellowfish individuals are caught on a regular basis.

2.3 Establishing radio telemetry methods

2.3.1 Radio tags

In this study, adult yellowfish were fitted with externally attached radio tags obtained from Wireless Wildlife International (WW) in Potchefstroom, North West Province, South Africa. These tags have been part of a developmental project and were therefore tested by personnel from Wireless Wildlife in a controlled environment. The tags were then again tested in the field before being attached to individual fish. Three types of tags were used, including:

WW-tag Series III – External fish mount tag with activity and temperature (Figure 9A) monitoring components. Total mass: 20 g ($^{+}$/-1.5g).

WW-tag Series V - External fish mount tag with activity, temperature and depth (Figure 9B) monitoring components. Total mass: 20 g ($^{+}$/-1.5g).

WW-tag Series VI - External fish mount transceiver with activity, temperature, depth and memory monitoring components (Figure 9C) to save data obtained while the WW-tag is not within transmission range. The stored data are then transmitted when connection to a receiver is established. Total mass: 20 g ($^{+}$/-1.5g).

The lifespan of the WW-tags currently exceeds 365 days (based on a battery life expectancy with an 80% safety factor) by combining default and tracking modes. Tags transmitting in default mode transmit every 10 min, whereas tags in tracking mode transmit every second. Monitoring scenarios available to all tags include:

Scenario 1: Default mode (transmission every 10 min) without any tracking modes results in a WW-tag lifespan of 20 months.

Scenario 2: Default mode (transmission every 10 min) with 40 h total manual tracking mode (transmission every second) results in a WW-tag lifespan of 12 months.

Therefore the tags were suitable to use and to monitor yellowfish individuals for one year. To obtain best results, three types of tags were allocated for this study; these included: five (WW-tag Series III), fifteen (WW-tag Series V), and one (WW-tag Series VI).

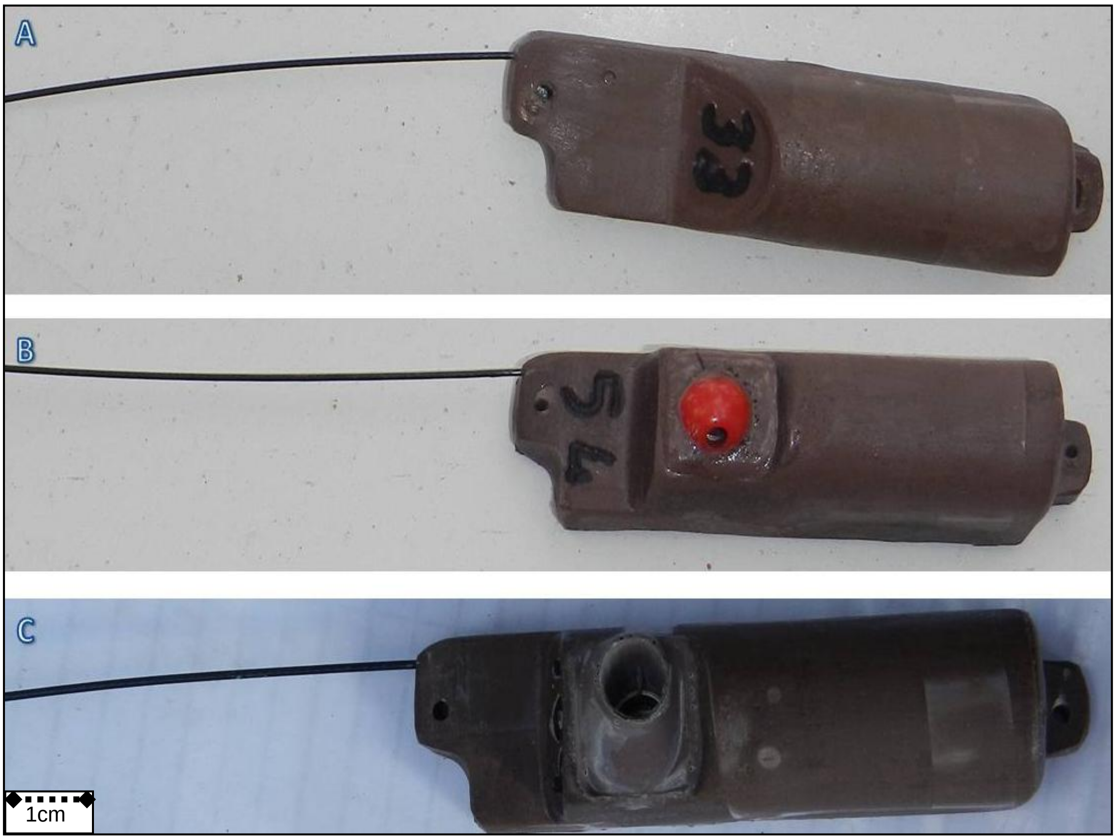

Figure 9: Different tags that were used in this study, including WW-tag Series III (A), WW-tag Series V (B) and WW-tag Series VI (C). A scale has been added for size.

2.3.2 Remote monitoring systems

Each study area had a remote monitoring system that consisted of one base station and a number of repeater stations (HAWK UHF-DL). They can be erected far apart to increase coverage area, with the only requirement being line of sight. Stations were all protected with activity and global positioning system (GPS) sensors. Activity sensors acted as an early warning if someone tampered or damaged the stations whereas GPS tracking sensors were used to recover stolen stations.

Remote monitoring stations consisted of five separate parts. These parts were assembled with a range of tools before stations were raised. It consisted of an Omni antenna (Figure 11A), a solar panel with the remote station (Figure 11B-C), and a cable that connect the antennae to the remote station (Figure 11D). For maximum height the antenna was connected to a length of angle iron that was connected to a wooden pole (Figure 11E-F). Remote monitoring stations were then attached to an existing structure such as a tree or building, or anchored in the ground and supported with concrete. Stations erected in trees and structures (Figure 11G) had an average height of 14 m while stations erected on the ground had an average height of 7 m (Figure 11H). The communication radius of tags to remote stations was approximately 1 km. Each station was identifiable, which assisted with theft, damage and malfunction issues. Station identification also contributed to identify the locality of fishes when a tag was in range of a remote monitoring station. Stations were allocated with code numbers starting from the base station as number one and then followed sequentially in a clockwise direction or in a downstream direction. The remote monitoring stations transmit data via GSM (cell phone) or radio networks to a server at Wireless Wildlife making use of a data-management system (Figure 10). Data can be downloaded or viewed from the data-management system using a password-protected Internet-based interface. Communication from tag to data-management system operates bi-directional, allowing users to change frequency of transmissions via a short message service (SMS) using a mobile telephone to the remote monitoring stations. By changing the transmission frequency of the tag (default transmits every 10 min) to tracking mode (transmits every second) it was easier to carry out manual tracking exercises. To change tag settings on fishes they had to be within range of a remote monitoring station for an extended time period.

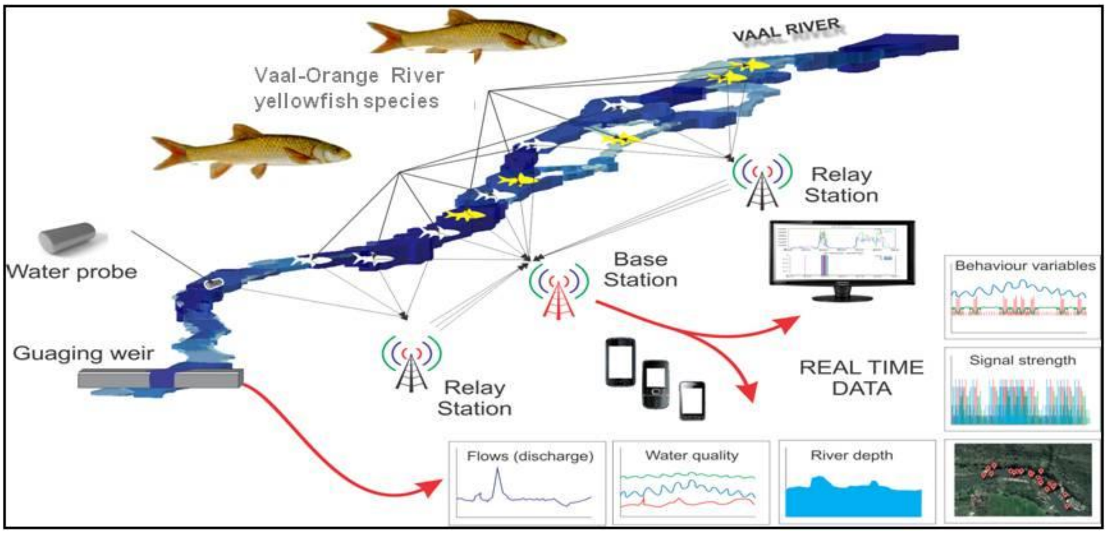

Figure 10: Diagram of the remote monitoring system, including signals from tags on individuals transmitted to remote monitoring stations around the study area; these data are then transmitted via a GSM network and can be accessed on a computer via the Internet

Figure 11: Assembly materials used for the remote monitoring stations: Omni antenna (A); solar panel with remote station (B-C); and a cable (D) that connects antennae and remote station (E-F). For extra height remote monitoring station was raised on any available structures such as trees (G-H).

Erecting remote monitoring stations at Boskop Dam

Possible locations for remote monitoring stations were identified using Google Earth and contour maps. Thereafter arrangements were made with all landowners for site inspections and to obtain permission to erect remote monitoring stations on their land if necessary. During site inspections a Garmin GPS (*E-trex®*) was used to obtain accurate locations for the stations. Binoculars were used to select the positions of other stations from the base station which required line of sight. This process was repeated until six locations were selected (Table 6). Remote monitoring stations in Boskop Dam were allocated with numbers starting with the base station as number one, and then numbering followed in a clockwise direction around the study area (Figure 12). Thereafter assembly materials were transported to each location, and stations were erected (Figure 13).

Table 6: Remote monitoring stations around Boskop Dam, including GPS position, allocated number, station code and land use

GPS position	Allocated number	Station code	Land use
26°32'16.57"S 27° 7'35.49"E	1	244	Private
26°33'38.15"S 27° 7'19.72"E	2	245	Private
26°34'5.30"S 27°6'51.74"E	3	251	Department of Water Affairs
26°33'43.78"S 27° 6'44.21"E	4	241	
26°31'53.53"S 27° 7'21.89"E	5	253	Boskop Dam Nature Reserve
26°32'44.80"S 27° 6'51.85"E	6	247	

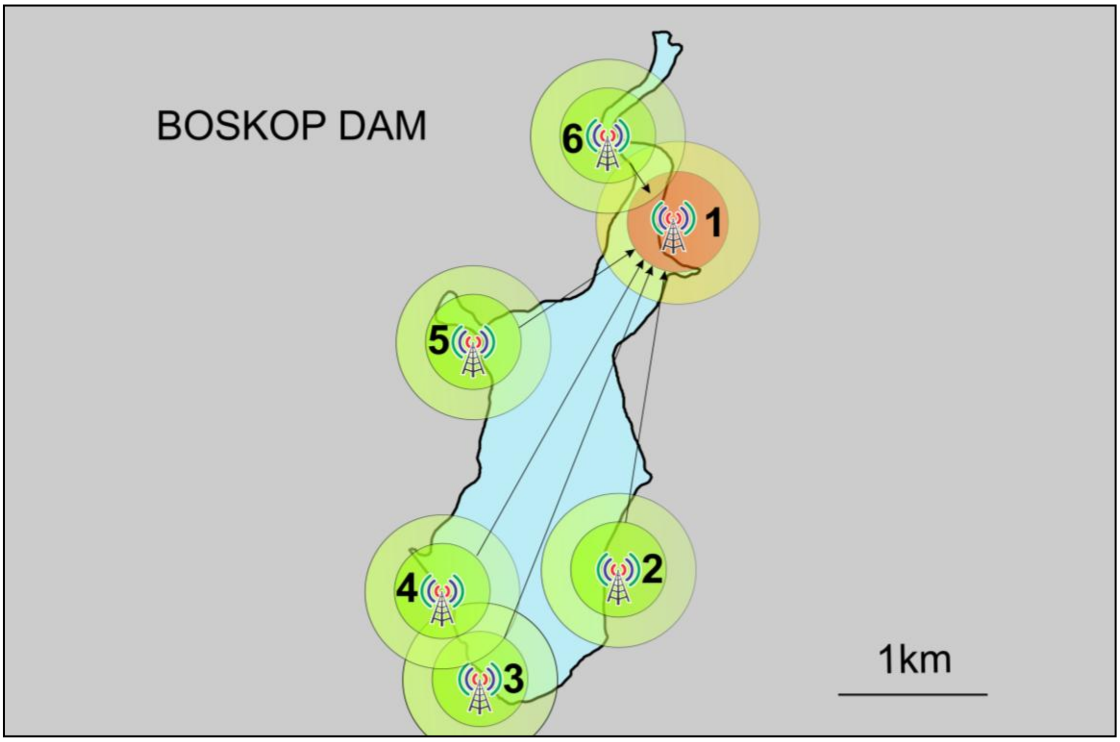

Figure 12: Map of remote monitoring stations around Boskop Dam: orange circle is the base station (1) and green circles are repeater stations (2-6)

Figure 13: Boskop Dam remote monitoring system, including one base station (1) and five repeater stations (2-6)

Erecting remote monitoring stations on the Vaal River

In this study area all remote monitoring stations were erected on one property (Wag ‘n Bietjie Eco Farm) along the Vaal River (Table 7). In order to have coverage of the entire study area, the first remote monitoring station was set up on an elevated water tank. From this position line of site access to the entire study area allowed for stations to be positioned, up to 5 km from the base station. Stations were mostly set up in trees to gain extra height for better coverage. Allocation of remote monitoring station numbers started from the base station as number one, and then the numbering followed from upstream of the study area downstream (Figure 14). Initially only four stations were set up on the Vaal River, but its effectiveness resulted in the addition of four more stations (Figure 15).

Table 7: Remote monitoring stations at the Vaal River, including GPS position, allocated number, station code and land use

GPS position	Allocated number	Station code	Land use
27° 9'37.96"S 26°27'10.04"E	1	249	Wag ‘n Bietjie Eco Farm
27° 7'53.23"S 26°29'4.83"E	2	242	
27° 9'18.02"S 26°27'5.09"E	3	253	
27° 9'1.42"S 26°26'25.50"E	4	255	
27° 9'1.85"S 26°26'3.57"E	5	245	
27° 9'8.27"S 26°25'39.51"E	6	251	
27° 9'16.65"S 26°25'33.89"E	7	243	
27° 9'47.55"S 26°25'25.46"E	8	247	

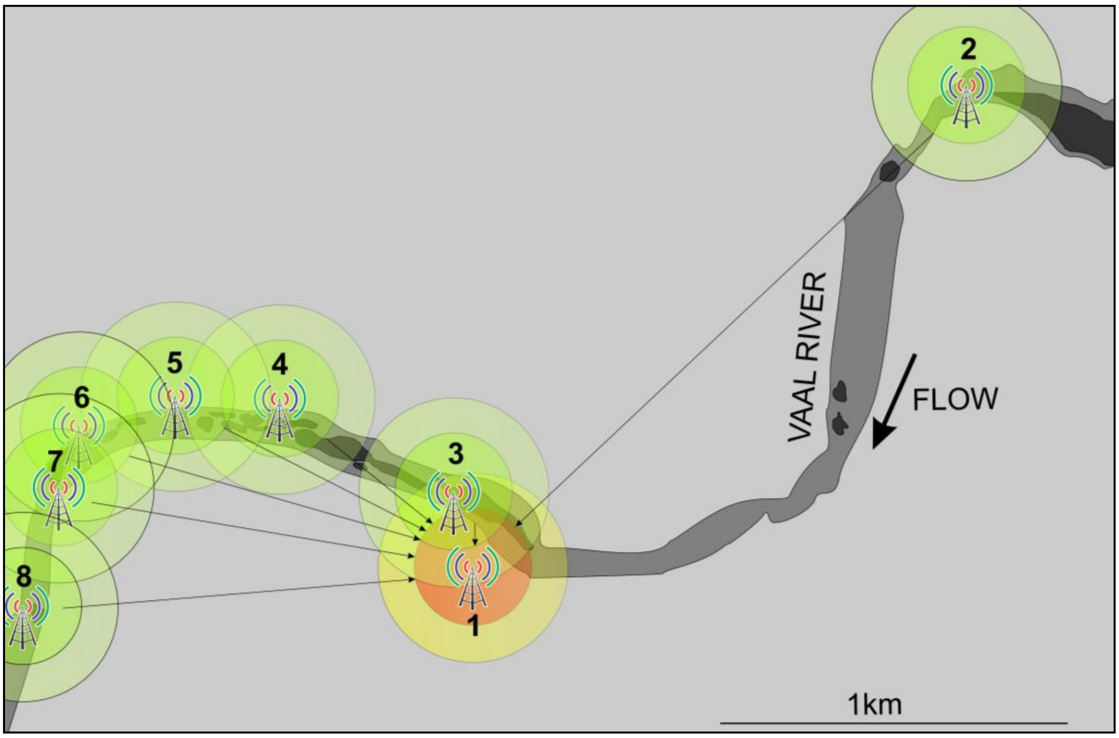

Figure 14: Map of remote monitoring stations on the Vaal River: orange circle is the base station (1) and green circles represent repeater stations (2-8)

Figure 15: The Vaal River remote monitoring system, including one base station (1) and 7 repeater stations (2-8)

2.3.3 Manual monitoring system

The manual tracking equipment consists of a programmable receiver, headphones and a directional Yagi antenna (Figure 18). The laptop receiver (Gigabyte model Q2005 incorporating Microsoft® Windows 7 operating system) or programmable mobile receivers connected to the directional Yagi antenna are used to monitor the location of tagged fish and associated behavioural information such as movement. It had the ability to show which tag was transmitting to which remote monitoring station. Numerous tags can be tracked simultaneously if in range with the laptop receiver. The mobile receiver can only be programmed to track a specific tag. Signal strength would then be displayed on the receiver, which gave an indication of the locality of the tagged yellowfish individuals in a specific coverage area. The programmable directional mobile receiver connected to the Yagi antenna could then be programmed to track a specific tag. Once the tag was in range the programmable mobile receiver would be used to send a setting to change the tag into tracking mode. Through triangulation signal and sound strength on the receiver the tagged individual could be pin-pointed accurately (Figure 17).

Figure 16: The receiver (GIGABYTE laptop) connected to the programmable mobile receiver attached to the directional Yagi antenna with headphones and data sheets

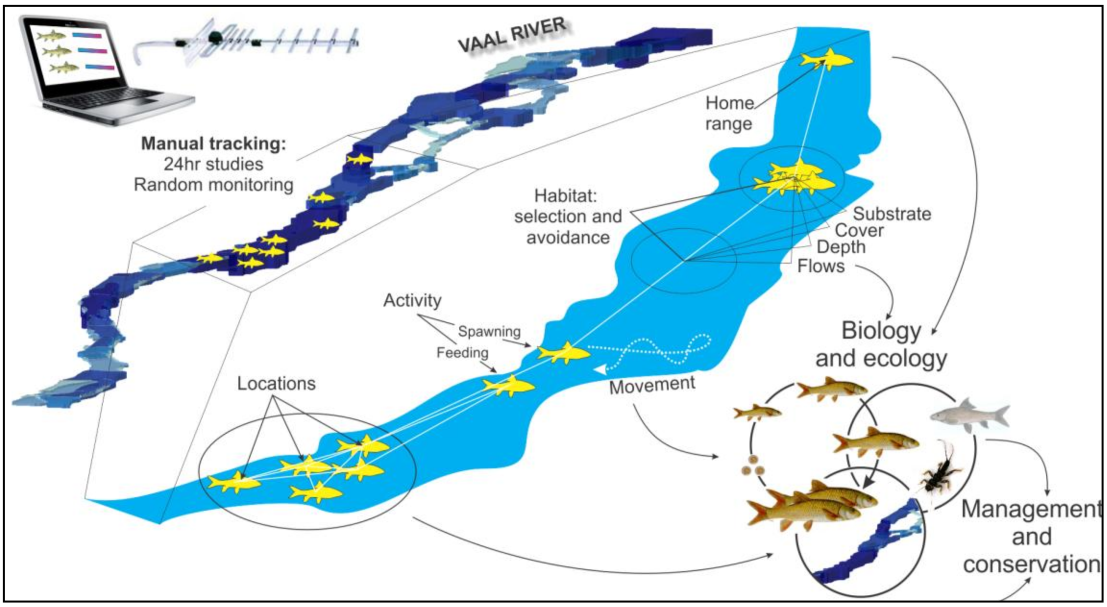

Figure 17: Diagram of the manual monitoring system. The receiver connected to the mobile programmable receiver attached to the directional Yagi antenna is used to monitor the location of tagged fish and associated behavioural information such as movement.

2.4 Environmental variables monitored

For this study, a number of environmental variables, including water flow, lunar cycles and different weather variables, were identified and monitored in order to assess whether they could possibly influence the behaviour of yellowfish. These variables were monitored at both study areas and assessed using a range of different techniques. Monthly environmental variables were recorded and divided into four seasons. Seasons were selected according to the normal South African seasonal calendar where September marks the beginning of spring; seasons were therefore divided as follows: spring (September, August, and November), summer (December, January, February), autumn (March, April, May) and winter (June, July, August).

Atmospheric variables including barometric pressure, rainfall and air temperatures were collected throughout the study using the closest possible weather station (Boskop Dam: C2R001Q01 UWQ) and (Vaal River: 04362041: Klerksdorp, South African Weather Service).

The influence of lunar cycles on yellowfish species has not been documented. Therefore this study used a normal lunar calendar and monitoring surveys were established according to the different lunar stages. Lunar stages were divided into full moon and new moon phases, where information from tagged individuals was recorded two days before, on the full or new moon, and two days thereafter. Therefore the information was gathered over five days for every full moon or new moon cycle.

Water flows were also monitored as an environmental variable as it changes habitat types, and behaviour of yellowfish species could be affected by volumes, timing and duration of flows. The South African Department of Water Affairs gauging station number (C2H007Q01 Vaal River at Pilgrims estate, Orkney) was used to estimate the water-quantity variables for the Vaal River study area. These changing habitat types were classified using Hirschowitz et al. (2007) and DWA (2010). These habitats included the use and/or availability of backwater areas, pools, glides, riffles, runs and rapids. In addition to consideration of these habitats, a few cover features included the use of and/or availability of undercut banks or root wads, dead and/or submerged trees, complex substrate types such as boulder beds, rocky outcrops and underwater ridges, marginal, aquatic and emergent vegetation, islands, water column and the top of or tail out of pools. The recording and scoring of habitat availability were aided by the use of three-dimensional digital terrain models of important reaches of the study area. These models were generated using ARC GIS®, from data that were either collected from a Hummingbird® 789CI side-scan fish finder or from manual observations identifying different depths, substrates and flows, and thereafter data were transferred to a computer for further analysis and to generate maps.

2.5 Capture, tag, release and monitor suitable yellowfish across four seasons

2.5.1 Fish collection

All methods used to capture yellowfish for this study were carefully evaluated to prevent unrepresentative sampling and biased statistics (Rogers and White, 2007). Suitable yellowfish included specimens that were large enough to carry a radio tag according to the 2% biotelemetry rule (Winter, 1996).Collection included the use of gill nets to target mobile individuals, in deep habitats, electro-fishing (electro-narcosis) to collect yellowfish in shallow habitats and angling techniques in a wide variety of habitats.

Netting techniques included large mesh gill nets (Figure 18A-C) that ranged between 93 mm and 120 mm. Large mesh sizes were selected to target only large individuals and minimize by catch. Gill nets were deployed in deep slow-flowing areas adjacent to suitable cover features and monitored until visible movement indicated that fishes had been caught. As soon as movement was observed fishes were immediately removed. Large fyke net traps (Figure 18D-E) were also used in areas with shallow, slow-flowing water to trap suitable individuals that could be used in the study. This involved deploying the traps in areas frequented by yellowfish and leaving them overnight. Two inflatable boats were used to transport nets and researchers around study areas (Figure 7F-G) and to access deeper water. Cast nets (Figure 18H-I) were also used in possible holding areas.

Electro-fishing or electro-narcoses were used as sampling methods for collecting yellowfish. The electro-fisher used in this study was a backpack electro-fisher (SAMUS725M) (Figure 18J-K). The anode carried by the person electro-fishing is inserted into the water which connects to a cathode trailing behind the individual. This connection creates an electrical stream with a 2 m radius approximately, depending on the conductivity of the water. Any fish within that radius will go into a state of narcosis (stunned). Stunned fish are then collected by means of a landing net.

Three types of angling disciplines were used in this study:

- Fly-fishing techniques (Figure 18L-N) where anglers use artificial flies made from synthetic material to represent natural food of yellowfish.
- Bait fishing (Figure 18O) with two or more hooks baited with worms, bread, sweet-corn or crabs. Suitable areas were selected, usually close to a current where rods would be rested on a tripod until a fish picked up the bait and jerked the line.
- The third angling discipline included the use of artificial lures (Figure 18P). This involved using lures made from balsa wood, iron or hard plastic (Rapalas®, Blue Fox spinners®, Action Lures®) to represent live swimming baitfish.

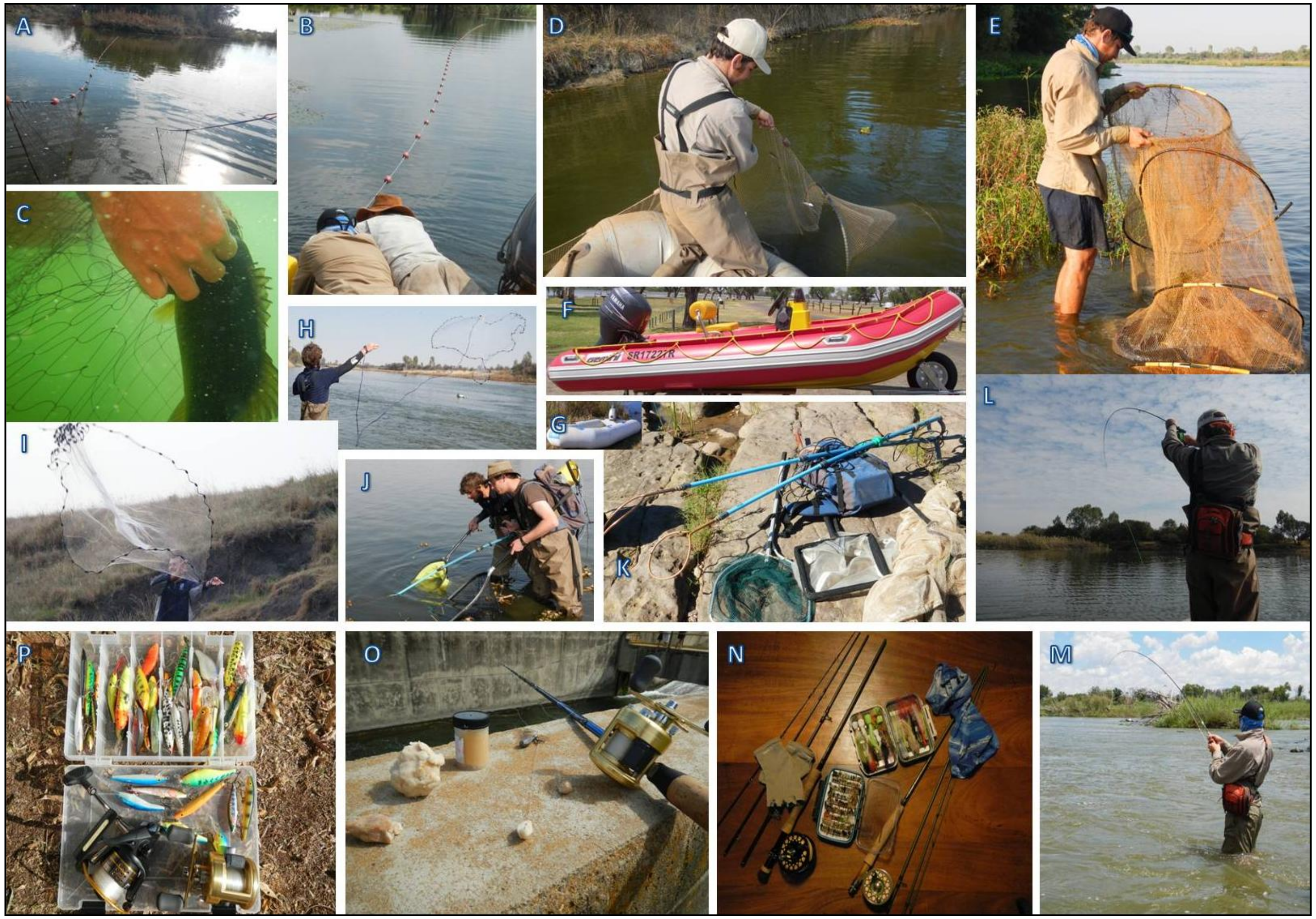

Figure 18: Methods used to capture yellowfish included: gill nets (A-C); fyke net traps (D-E); boats used (F-G); cast nets (H-I); electro-fishing (J-K); fly-fishing (L-N); bait fishing (O); and artificial lure fishing (P)

2.5.2 Radio tagging

The radio tags used in this study were mounted externally. Although this method is known to have the potential to imbalance the tagged fish and has a high fouling potential, this method has been proven to be successful on yellowfish (O'Brien *et al*., 2013). In addition, the two percentage rule of tag mass to fish mass was maintained which has proven successful for use on fish in freshwater ecosystems (Knights and Lasee, 1996; Winter, 1996; Koehn, 2000). For this study a collapsible tagging station was developed. Advantages of having a collapsible tagging station included:

- Fish could be tagged where captured (Figure 19A).
- No out-of-water transport from one point to another required.
- Water from the same area is circulated through container.
- Fully submerged fish usually kept calm (Figure 19B).
- Correct amount of anaesthetic was added every time (Figure 19C).
- Holding time of fish was kept to a minimum.
- Fish was never taken out of water and tagged while fully submerged (Figure 19D).
- Close-up inspection and treating of fish diseases was possible (Figure 19E).
- System consists of only a few parts (battery, bilge pump and tagging kit) (Figure 19F-H).
- Fish could easily be measured.
- Tagging could be done quickly and effectively.
- Very little physical handling of fish was necessary.
- Fish could be fully revived in the container before being released back into its environment (Figure 19I).

When a suitable yellowfish was captured it was immediately transferred to the collapsible tagging container. Care was taken to keep fish in water at all times and as a rule, little or no touching was practised. To begin the tagging process (approved by the North West Ethics Committee NWU-00095-12-A4) the out flowing tap on the container was closed and the bilge pump supplying fresh water was disconnected. Thereafter 10 ml of a pre-mixed bottle containing 2-phenoxy ethanol (0.4 ml/l) was added to the still standing water, until signs of narcosis became evident.

Figure 19: Collapsible tagging station included advantages such as: fish tagged were captured (A); preparations made while fish totally submerged and calm (B); correct amount of anaesthetic always added (C); fish tagged in water (D); close-up inspection and treatment of fish diseases (E); station consists of only a tagging kit, battery and bilge pump (F-H); and fish can be fully revived before being released (I)

Signs of narcosis included: operculum movement slowed down, fish became sluggish and if left any longer, fish turned over (Figure 20A). As soon as any signs of narcosis became evident, the tap on the container was immediately opened, releasing the water containing the anaesthetic while the bilge pump supplying fresh water was reconnected.

Tagging equipment was cleaned in ethanol before use. In the anaesthetised state, two surgical needles were pushed through the musculature of the individual yellowfish at the base of the dorsal fin (Figure 20B-C). Nylon lines with plastic stoppers at one end were then threaded through the surgical needles (Figure 20D). Thereafter needles were slowly removed (Figure 20E). The tag was attached by inserting the nylon through the holes of the tags and seated firmly against the fish (Figure 20F). Crimping pliers were then used to crimp the copper sleeves on the nylon, and to cut off the excess nylon to make it neat (Figure 20G-H). An antibiotic (Terramycin® containing oxy-tetracycline) was then injected in the muscle at a concentration of 1 ml/kg (Figure 20I), Betadine was used on areas where fish had been touched (Figure 20J), and wound-care gel (Aqua Vet) was applied to wounds (Figure 20K) to treat and minimise risk of infections. After tagging measurements (TL, FL, SL and girth) and mass (g) had been recorded, the tagged yellowfish was left in the circulating water in the container until it had fully recovered (Figure 20L). Thereafter photographs of the fish were taken (Figure 20M) in a semi-narcotic state and after full recovery the fish was safely released back into the system (Figure 20N-O).

Figure 20: Tagging process following sedation (A). Two surgical needles were pushed through the muscle at the base of the dorsal fin (B-C), thereafter nylon line with plastic stoppers was threaded through the needles (D). Needles were then slowly removed (E); nylon line was then put through holes on tag until tag sat firmly (F); crimping pliers were used to crimp the copper sleeves (G); and side-cutters cut excess nylon (H); Terramycin, Betadine and wound-care gel are used to treat and prevent infections (I-K); yellowfish fully revived (L); quick picture was taken (M); and fish released back into system (N-O).

2.5.3 Tracking and monitoring

Yellowfish individuals were monitored directly after tagging, to establish behavioural response to tagging and to ensure the survival of the tagged fish. In this study 24 h were allocated to the recovery process of the tagged fish following anaesthesia and attachment procedures (Bridger and Booth, 2003). Thereafter tracking and monitoring of the tagged yellowfish individuals were carried out at scheduled and random intervals. Scheduled surveys were established according to the lunar cycle, where surveys would take place on full moon and new moon phases predominantly (Table 8). The random surveys were carried out throughout the study period and were used to tag fishes, repair equipment and document behaviour in events such as cold fronts, rainfall and sudden changes in water flows.

Table 8: Surveys carried out throughout the study, including study area, specific or random intervals, season, month, survey dates, moon phases and aim of surveys

Study area	Specific/ random	Season	Month	Survey dates	Moon phase	Aim
Boskop Dam	Random	Summer	Sep-11	6-7	First quarter	Erect remote monitoring stations
Boskop Dam	Random	Summer	Oct-11	5-6	First quarter	Tagging
Boskop Dam	Specific	Summer	Nov-11	4-16	Full moon	Tagging
Boskop Dam	Specific	Summer	Jan-12	23-30	New moon	Tagging
Vaal River	Random	Summer	Feb-12	10-12	Last quarter	Erecting remote monitoring stations
Vaal River	Random	Summer	Feb-12	15-16	Last quarter	Tagging
Vaal River	Specific	Summer	Feb-12	20-21	New moon	Tagging
Vaal River	Specific, 24 h	Summer	Feb-12	25-27	New moon	Document behaviour in rainfall/flow changes
Vaal River	Random	Summer	Feb-12	27-29	First quarter	Tracking and monitoring/Tagging
Vaal River	Random	Summer	March	14-16	Last quarter	Tracking and monitoring/tagging
Boskop Dam	Random	Autumn	May-12	23	New moon	Maintenance check
Vaal River	Random	Autumn	May-12	24	New moon	Maintenance check
Vaal River	Random	Autumn	May-12	30	First quarter	Erect new remote monitoring stations
Vaal River	Specific	Winter	Jun-12	19-23	New moon	Tracking and monitoring/tagging
Vaal River	Specific	Winter	Jul-12	3-5	Full moon	Tracking and monitoring/tagging
Vaal River	Specific	Winter	Jul-12	18-20	New moon	Tracking and monitoring/tagging
Vaal River	Random	Winter	Jul-12	26-27	First quarter	Cold front
Vaal River	Specific, 24 h	Winter	Aug-12	1-3	Full moon	Cold front
Vaal River	Specific	Winter	Aug-12	13-15	New moon	Tracking and monitoring/tagging
Vaal River	Random	Spring	Sep-12	7-9	Last quarter	First summer rains/flow changes
Vaal River	Random	Spring	Sep-12	29-30	Full moon	Document spawning

Tracking and monitoring surveys were initiated by setting up manual monitoring equipment (Figure 21) at the study area, in range of any remote monitoring station. The receiver would then display which tags were transmitting to which remote monitoring station. If a tag transmits frequently (transmits every 10 min) to a specific remote monitoring station, transmitting frequency could be changed to tracking (transmitting every second) using the SMS system. Alternatively the programmable mobile receiver was programmed to change the transmitting frequencies of a tag, if in range of the tag.

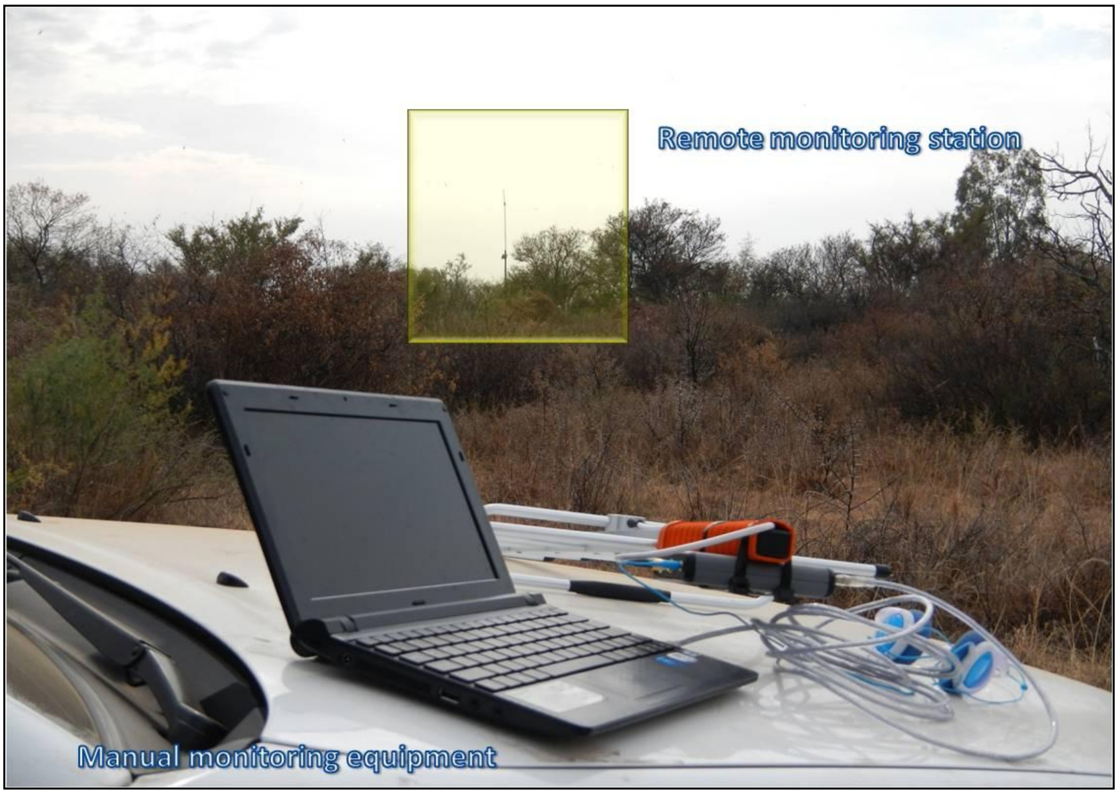

Figure 21: Manual monitoring equipment set up in range of remote monitoring station

Once the transmitting frequency of the tag has been changed (tag number displayed in green block) (Figure 22) the exact position (1 m accuracy) of the tagged fish could be determined. To identify exact position of the tagged fishes, the person tracking began searching from the remote monitoring station, to which the tag transmitted. From there the receiver connected to the programmable directional Yagi will be in range of the transmitting tag; signal strength is then displayed on the receiver and audio sounds through headphones.

The receiver picked up tagged fishes from a distance of about 500 m depending on the depth of the tagged fish. From there the position of tagged fishes could be accurately identified by walking (Figure 22A) or drifting in a boat (Figure 22B), following signal strength and sound. When a tagged fish was located monitoring

(Figure 23A-B) with 10 min intervals for 40 min were initiated. To accurately locate tagged individuals signal strength was used. Signal strength became stronger (red to orange and then yellow) (Figure 24A-C) and sound pitch becomes higher as a tagged fish was approached. When signal strength is at its strongest (green) (Figure 24D) and sound pitch is maximised, a positive location of a tagged fish was identified. At each fix the following data were recorded on data sheets: date and time; tag number; location (obtained from geo-referenced maps on a Trimble (Geo-explore or hand-held GPS *eTrex*); movement (maximum displacement per minute (MDPM)); habitat types associated with location; weather variables; noted sketches of yellowfish movement; any other fish activity; disturbance; predators; insect hatches; and any other information that would be available at a specific area.

Figure 22: Researcher identifying position of tagged fish, either by walking on the bank (A) or drifting in a boat (B)

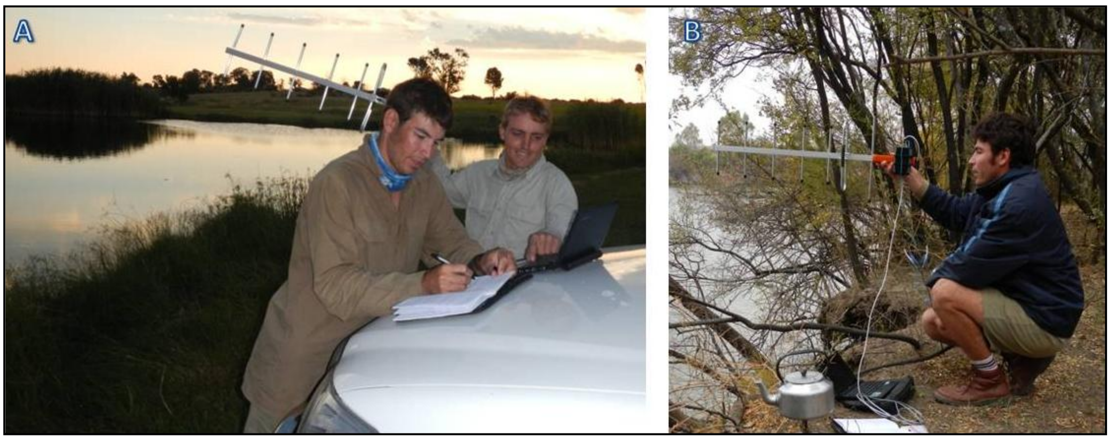

Figure 23: Behaviour of tagged fishes being monitored and documented

Figure 24: Signal strength displayed on receiver approaching a tagged fish, including weak red signal (A); orange (B); yellow (C); and finally green (D) indicating that signal strength is strongest, and exact position can be identified

2.6 Statistical evaluation of yellowfish behavioural data collected throughout the study

In this study the movement of the yellowfish was selected as the behavioural variable used to evaluate the effect of changing environmental variables on the test organisms. Movement data included MDPM obtained during manual tracking events and movement counts per minute (MC/min) of individuals using remote systems. Variables considered included seasons, time, tag number, activity of the fish, associated substrate, habitat, weather, and moon phases. In addition, the depth and temperature recordings from the tags were used; these data were downloaded from the data-management system in a *.csv file format. The water quality and flow (measured as discharge) variables, lunar cycles and different atmospheric weather variables presented above were also considered.

Spatial and temporal trends were analysed using ARC GIS®. Using this approach each individual's spatial area use could be evaluated, including high area use, preferred areas and the relationships between location and environmental variables (Hodder *et al.,* 2007). Movement and depth were calculated using box-and-whisker plots where estimates are based on 25th and 75th percentiles while whisker extremes are based on 5th and 95th percentiles. Relationships between the movement of yellowfish species in MDPM and changes in the environmental conditions were statistically analysed using the approach adopted by O'Brien *et al*. (2013). This approach used a mixed-model analysis of variance (ANOVA) together with a coefficients model (Littell *et al*., 1996) and Akaike's Information Criteria (AIC) (Burnham and Anderson, 1998) and data were statistically analysed and significant values ($P<0.05$) were calculated by the Statistical Consultation Services of the North West University in Potchefstroom using SAS Version 9.3 (SAS Institute, Cary, NC).