MALOTI MINNOW SURVEY

MOHALE AND EASTERN ESU

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Abstract

A fish distribution survey was undertaken in the Lesotho Highlands from 31 July – 16 August 2017 to re-assess the status of both evolutionary significant units of the Maloti minnow *Pseudobarbus quathlambae*. A total of 13 rivers and 42 sites were surveyed. *P. quathlambae* (Mohale ESU) is no longer present in the Senqunyane, Bokong and the Jorodane River (below Pampiri Falls) upstream of Mohale Dam. The disappearance of *P. quathlambae* from these primary habitat rivers is most likely a result of predation by and competition for habitat and food from smallmouth yellowfish *Labeobarbus aeneus*. The construction of a barrier in the Senqunyane to protect *P. quathlambae* is no longer necessary. Unless a programme focussing on the eradication of *L. aeneus* and restocking of *P. quathlambae* is initiated, construction of a barrier at this stage would serve no purpose.

Populations of translocated *P. quathlambae* (Mohale ESU) were recorded in the Jorodane- (above Pampiri Falls) (n = 38), Makhaleng- (n = 11), and Maletsunyane River (n = 41). No fish were recorded in the Quthing River.

P. quathlambae (Eastern ESU) was recorded in the Tsoelikane- (n = 15), Sani- (n=2), Mothae- (n = 7), upper Matsoku- (n = 33), Senqu- (n = 15) and Moremoholo River (n = 33). The Maloti minnow is not extinct below the Tsoelikane Falls as previously thought. Very few *P. quathlambae* were recorded in the Sani- and Mothae River and none were present in the lower Matsoku River.

No rainbow trout (*O. mykiss*) were recorded in any of the rivers surveyed.

The health and size of various minnow populations cannot be accurately determined at this stage. It is recommended that a follow-up survey be conducted in early summer to better understand the population dynamics of the translocated populations and the Eastern ESU populations. These surveys should incorporate river health and rangeland assessments to provide baseline data for future monitoring of the populations. Once the status of the populations has been established then a new management plan and conservation actions must be prepared and implemented. This should include a monitoring programme which incorporates aquatic and terrestrial components. It is recommended that a workshop is held to develop a roadmap for the conservation of *P. quathlambae* in the Lesotho Highlands.

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List of Acronyms and Abbreviations

BMU – Biodiversity Management Unit

CPUE – Catch per unit effort

ESU – Evolutionarily significant unit

FSL – Full supply level

IBT – Inter-basin transfer

IUCN – International Union for Conservation of Nature

KZN – Kwazulu-Natal

LHDA – Lesotho Highlands Development Authority

LHWP – Lesotho Highlands Water Project

MU – Management Unit

PERMANOVA - Permanent multivariate analysis of variance

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1. Introduction

The Maloti minnow *Pseudobarbus quathlambae* (Figure 1) is a small cyprinid (< 130mm total length (TL)) endemic to the highlands of Lesotho (Cambray, 1996) and the KZN Drakensberg (Barnard, 1938). It was considered extinct in South Africa until recently when it was rediscovered in several localities that have not been disclosed at this stage (Albert Chakona, personal communication, September 2017). Its favoured habitats include pools and sheltered areas in clear, low to moderate gradient streams on basalt or sandstone (Cambray and Meyer, 1988; Rall et al., 1993; Skelton, 2001). *P. quathlambae* has a high mortality rate (Z = 2.47) with a recruit survival rate of 8% at the end of the first year and a maximum age of four years (Cambray and Meyer, 1988). Aquatic invertebrates, including nymph and adult mayflies (Ephemeroptera), true flies (Diptera), and black fly (Simuliid) larvae, form the bulk of the diet (Cambray, 1996). *P. quathlambae* are serial spawners with spawning occurring a number of times during the rainy season (late October to February) (Cambray and Meyer, 1988).

Figure 1: The Maloti minnow (*Pseudobarbus quathlambae*)

P. quathlambae is comprised of two genetically distinct sub-populations or evolutionarily significant units (ESUs): an “Eastern ESU” viz. *P. quathlambae* and a “Mohale ESU” viz. *P. quathlambae* cf. Mohale (Swartz, 2005). The Eastern ESU occurs in several rivers of the Senqu catchment, while the Mohale ESU occurs in the upper reaches of the Senqunyane-, Bokong- and Jorodane River within the Mohale Dam catchment (Swartz, 2005) (Figure 2). The two ESUs are confined to only nine rivers and their distribution has become increasingly fragmented and limited due to habitat degradation, habitat loss (as a result of Phase 1B of the LHWP), and competition and predation from non-native (Shelton et al., 2016) as well as native fishes (McCafferty et al., 2017).

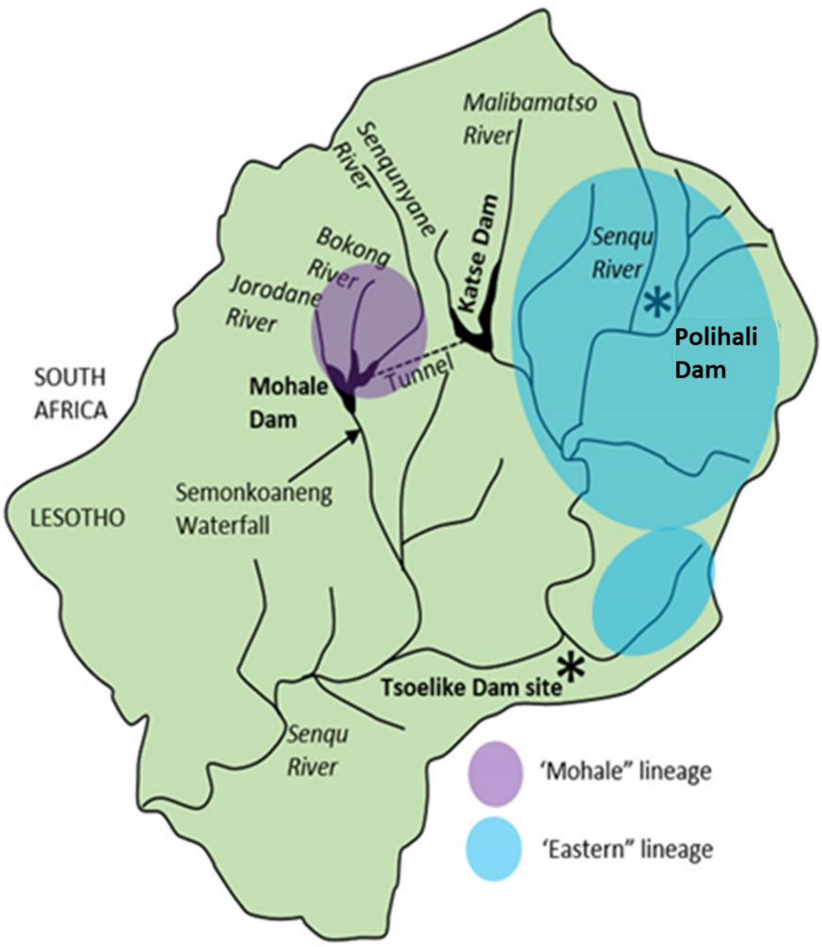

Figure 2: Distribution of the Mohale ESU and the Eastern ESU in the Lesotho Highlands (Shelton et al., 2016)

1.1 The Mohale ESU

The Mohale ESU is classified as "critically endangered" in the IUCN Red List of Threatened Species (Swartz, 2007). Historically, *P. quathlambae* was the only fish species that occupied the rivers upstream of Mohale Dam as the Semonkoaneng waterfall acts as a barrier to upstream migration of smallmouth yellowfish *Labeobarbus aeneus*, Orange River mudfish *Labeo capensis*, rock catfish *Austroglanis sclateri*, rainbow trout *Oncorhynchus mykiss*, and brown trout *Salmo trutta* (Steyn et al., 1996). In 2003, a 32km long inter-basin transfer (IBT) tunnel was constructed, which links Mohale Dam to Katse Dam.

Steyn et al. (1996), Rall (1999), and Skelton et al. (2001) predicted that fishes from Katse Dam, comprising *L. aeneus*, *L. capensis* and *O. mykiss*, would colonise Mohale Dam following the construction of the IBT tunnel, potentially placing *P. quathlambae* at risk of predation and/or competition, particularly from *O. mykiss.* The "Maloti Minnow Conservation Project" (Skelton et al., 2001) was a comprehensive, multi-disciplinary study, which developed a conservation action plan and recommended several conservation measures for the Mohale ESU. These were;

- Construction of artificial barriers
- Design and construction of holding and breeding facilities
- Translocation of *P. quathlambae*
- Design and implementation of a monitoring network
- Proclamation of reserves
- Review of the legal and institutional frameworks and guardian institutions
- Public participation, training and education

The construction of physical barriers across the Senqunyane-, Bokong- and Jorodane River to prevent the invasion by *O. mykiss* and *L. aeneus* (that would inevitably colonise Mohale dam through the IBT) and the design and construction of holding facilities were flagged for immediate implementation in 2001, while translocation was flagged for implementation in the short term and public participation, training and the establishment of reserves were identified for medium term implementation.

However, further studies suggested that any barrier across the Bokong- and Jorodane River would serve no purpose. The reasons being that the Bokong River was considered to be marginal for *O. mykiss* (shallow with daily temperature fluctuations of up to 17°C and a maximum recorded temperature of 32°C). A barrier on the Jorodane River, even if constructed immediately upstream from Full Supply Level (FSL) of Mohale Dam would add a very short reach between FSL and Titi Waterfall and was therefore not regarded as beneficial (Dr J. Rall, pers. comm. 2011).

In 2002/3 a total of 1700 *P. quathlambae* were translocated to the Quthing-, Makhaleng-, Maletsunyane- and Jorodane River (upstream of the Pampiri Falls). These fish were captured from the Senqunyane-, Bokong- and Jorodane River (Rall, 2005). Except for the fish translocated into the Quthing River, Rall and Sephaka (2008) found that viable (and breeding) populations had established themselves in the other three rivers.

In 2004, a survey confirmed that *L. aeneus* and *L. capensis* had successfully invaded Mohale Dam via the IBT tunnel and a follow-up survey in 2006 showed that *L. aeneus* had become more abundant in Mohale Dam, but had not penetrated the inflowing rivers. *P. quathlambae* was the only species recorded in the inflowing rivers (Rall and Sephaka, 2008). The authors warned that an invasion of the Senqunyane River was "imminent" and recommended the construction of a meander cut. In 2011, a gillnet survey of Mohale Dam showed that *L. aeneus* had become extremely abundant in the dam. However, evidence of upstream invasion of the inflowing rivers by yellowfish was, at this stage, only anecdotal (Hecht, 2011). Similarly, there was hearsay but no confirmed records of rainbow trout in Mohale dam (Hecht, 2011).

In 2013, a comprehensive electrofishing survey of the Jorodane, Bokong, and Senqunyane River revealed the presence of *L. aeneus* in all three rivers (Shelton et al., 2016). Furthermore, only five *P. quathlambae* were recorded during that survey, 3 in the Bokong and 2 in the Jorodane and none in the Senqunyane River, a previous stronghold of the Mohale ESU (Shelton et al., 2016). Shelton et al. (2016) concluded that the Mohale ESU was on the verge of extinction.

The Lesotho Highlands Development Authority's (LHDA) Biodiversity Management Unit (BMU) conducted a survey of the Senqunyane River in May 2017 (McCafferty et al., 2017). The aim of the study was to assess the status of *P. quathlambae* and non-native *L. aeneus* and *L. capensis* populations with a view to re-evaluating the relevance of constructing a physical barrier on the Senqunyane River. No *P. quathlambae* were recorded at eleven sites while *L. aeneus* was recorded at seven of the 11 sites. It was concluded that there is no longer a viable population of *P. quathlambae* in the Senqunyane River and that the construction of a barrier would not serve any purpose. It was emphasised that there was a need to re-assess the status of native *P. quathlambae* populations in the Bokong- and Jorodane River (below the Pampiri Falls) and the status of translocated populations in the Jorodane- (above the Pampiri Falls), in the Makhaleng-, Maletsunyane- and Quthing River. The findings of this survey would provide the decision support to decide finally on whether to build a barrier or not (McCafferty et al., 2017).

1.2 The Eastern ESU

Comparatively less work has been undertaken on the Eastern ESU. This is probably a result of the isolation of the Eastern ESU populations (with the exception of the Matsoku River population) from impacts associated with the Lesotho Highlands Water Project (LHWP). The first record of *P. quathlambae* Eastern ESU (then *Oreodaimon quathlambae*) was from the Tsoelikane River in the Sehlabathebe National Park in 1970 (Pike and Tedder, 1973). In 1973, 56 fish were translocated above the Tsoelikane waterfall to protect this population from *O. mykiss* below the falls (Pike and Tedder, 1973). Subsequently, populations of *P. quathlambae* were discovered in the Senqu- and Moremoholo Rivers in 1975 (Rondorf, 1976), the Sani River in 1988 (Skelton, 2000), and the Matsoku River in 2000 (Swartz, 2005).

In 2001, a survey of these populations was conducted as part of the Maloti Minnow Conservation Project (Skelton et al., 2001) (see Section 1.1.). The major outcomes from that study were as follows:

- Populations of *P. quathlambae* in the Matsoku-, Senqu-, Moremoholo-, Sani- and Tsoelikane River should be recognised as the Eastern ESU.
- The Eastern ESU comprises four management units (MUs):
    1. Matsoku MU
    2. Senqu – Moremoholo MU
    3. Sani MU
    4. Tsoelikane MU
- Each MU contributed a significant proportion to the total genetic diversity of the Eastern ESU. The loss of an MU would therefore be considered a major threat to the survival of *P. quathlambae*.
- Major threats to the Eastern ESU included non-native fish, specifically trout, and habitat degradation
- Conservation measures considered included a trout eradication programme and river restoration and habitat reclamation plans. This included the development of a management plan aimed at zoning different rivers as “conservation” rivers or as sport fishing rivers.

The Maloti Minnow Conservation Project was the last comprehensive survey of the Eastern ESU populations. In 2011, a previously unrecorded population of *P. quathlambae* was discovered in the Mothae River, a tributary of the Matsoku River (Paxton, 2011). The presence of a range of size- and age classes indicated that the population was healthy and recruiting. However, the development of a diamond mine in the immediate area and extensive mining activity in the region was cause for concern, specifically increased sediment loading, wastewater pollution and water abstraction (Paxton, 2011).

In summary, the threats faced by the Eastern ESU have historically been non-native fish, specifically trout, as well as habitat degradation. Phase II and Phase III of the LHWP will see the construction of Polihali dam and Tsoelike Dam, on the Senqu- and Tsoelikane River catchments, respectively. An impact assessment of the proposed Polihali Dam on fish populations in the Senqu and Moremoholo Rivers was conducted in 2014 (Bok, 2014). It was concluded that the dam would have no direct impact on *P. quathlambae* populations in the Senqu and Moremoholo Rivers as both occurred "well above the zone of influence" of the project. The populations were protected from trout and yellowfish moving upstream from the dam by "high waterfalls" (Bok, 2014). However, the populations of *P. quathlambae* above these waterfalls were not surveyed and a comprehensive assessment of the suitability of the waterfalls to act as barriers to invasion was not conducted.

Given the consequences of Phase 1 on the Mohale ESU, and a lack of recent research on the Eastern ESU, it is essential to undertake a study of the population status of the Eastern ESU populations. This will guide future research requirements and development of appropriate conservation plans.

1.3 Objectives

The objectives of this study were:

1. Re-assess the status of **native** and **translocated** populations of *P. quathlambae* Mohale ESU.
2. Re-assess the status of *P. quathlambae* Eastern ESU populations.
3. Re-evaluate the relevance of constructing a physical barrier across the Senqunyane-, Bokong- and Jorodane River.
4. Make recommendations for the conservation of both the Mohale ESU and the Eastern ESU.

2. Methods

The locations of the survey sites are shown in Figure 3.

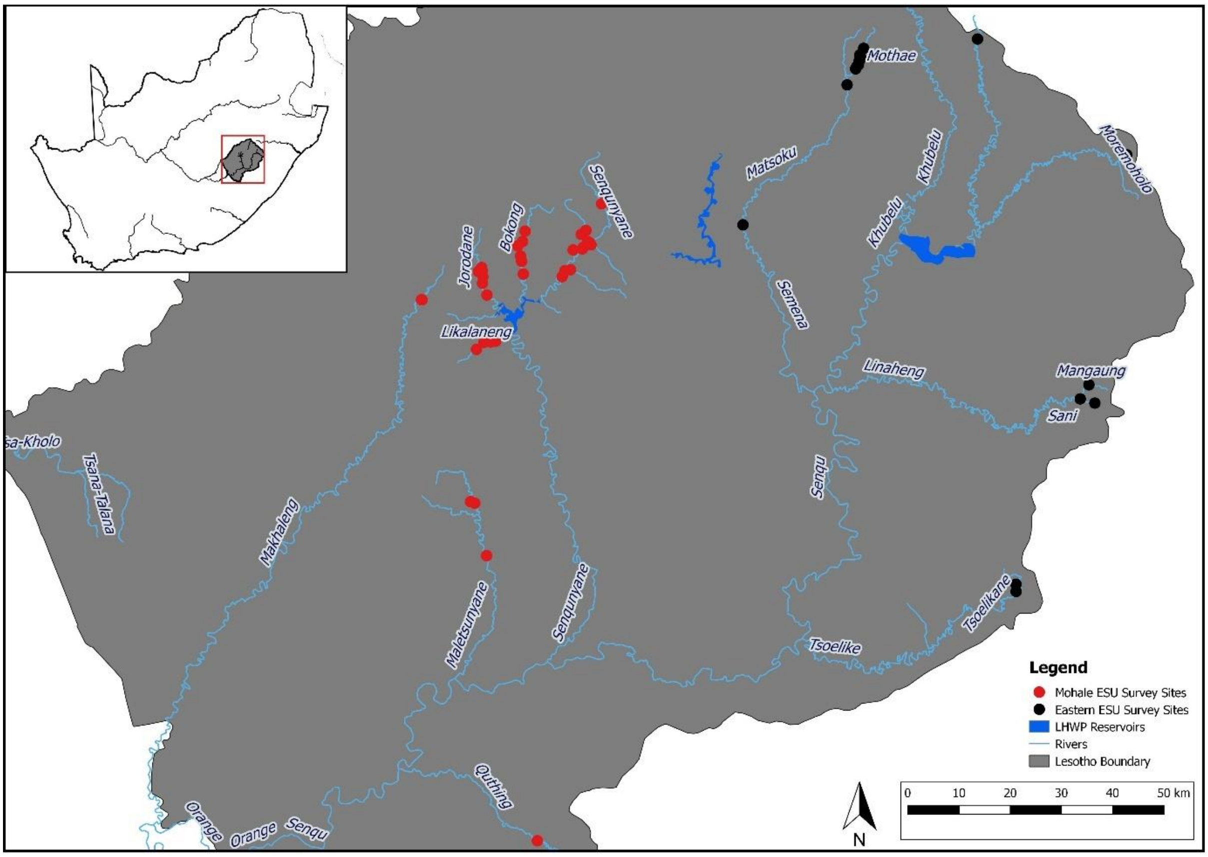

Figure 3: Survey sites for the Mohale ESU and the Eastern ESU, August 2017.

2.1 Mohale ESU Survey sites

The Mohale ESU survey was undertaken from 31 July to 6 August 2017 and 15-16 August 2017. A total of 25 sites were surveyed. The location of sites on the Senqunyane-, Bokong-, Jorodane- (below Pampiri Falls) and Likalaneng River was identical to those surveyed by Steyn et al (1996), Rall (1999), Rall and Sephaka (2008) and Shelton et al. (2016). Sites DT5 (Makhaleng River), JR9, JR8, DT6 (Maletsunyane River), and JR19 (Quthing River) were identical to those surveyed by Skelton et al (2001) and Rall and Sephaka (2008). Sites JT5, JT4, JT3, JT2, JT1 (Jorodane River) and MAK2 (Makhaleng River) were selected by the survey team on the basis that they were representative of at least two of the dominant biotopes observed (Table 1). (See Appendix A for maps of each river with sampling sites; photos of all sampling sites are captured in a database).

Table 1: Survey sites for the Mohale ESU, August 2017. (Shaded grey area indicates sites surveyed in May 2017).

Mohale ESU			
River	**Site**	**Location**	**GPS Coordinates**
Senqunyane	S29.3	Ha Mothakathi, upper Seipone	S29.27247; E028.24284
Senqunyane	S29.2	Ha Mothakathi, Tenteng	S29.27954; E 028.23341
Senqunyane	S29.1	Ha Mothakathi, Seipone	S29.29195; E 028.24657
Senqunyane	S29	Ha Mothakathi	S29.29451; E028.25093
Senqunyane	S28.2	Ha Ra Tsosane, upper Ntiboho	S29.29765; E028.25324
Senqunyane	S28.1	Ha Nnokoane lower Ntiboho	S29.30214; E028.23790
Senqunyane	S28	Ha Nnokoane	S29.30505; E028.23596
Senqunyane	S25.2	Ha Maime, Khohlong	S29.30709; E028.21746
Senqunyane	S25.1	Ha Mokhobi, Tsoelike	S29.34195; E028.21253
Senqunyane	S25	Ha Thaba-Bosiu	S29.34406; E028.20098
Senqunyane	S24	Ha Thaba-Bosiu	S29.35438; E028.19587
Senqunyane	S34	Ha Ramabele	S29.22528; E028.27378
Bokong	B8	Ha Motipi Kopano	S29.27486; E028.12063
Bokong	B7	Ha Motipi Kopano	S29.2932; E 028.11566
Bokong	B6	Ha Sebatalali	S29.30085; E 028.1058
Bokong	B5	Moeling	S29.31878; E028.11187
Bokong	B4	Ha Koko Raloti	S29.32806; E028.11413
Bokong	B3	Ha Paepae	S29.3504; E028.11782
Jorodane	JT5	Ha Lira	S29.33946; E028.03371
Jorodane	JT4	Ha Lira	S29.34210; E028.03279
Jorodane	JT3	Leropong	S29.34505; E028.03509
Jorodane	JT2	Leropong	S29.34673; E028.02725
Jorodane	JT1	Pampiri Falls	S29.35576; E028.03630
Jorodane	J8	Pampiri Falls	S29.35040; E028.11782
Jorodane	J7	Ha Rapokoloane	S29.36733; E028.0358
Jorodane	J6	Ha Likomisi	S29.38793; E028.04498
Likalaneng	L5	Khoshane	S29.48484; E028.02483
Likalaneng	L4	Tiping	S29.47134; E028.03983
Likalaneng	L3	Ha Mohale	S29.47078; E028.05308
Likalaneng	L2.5	Ha Mohale	S29.46927; E028.06339
Makhaleng	DT5	Qiloane Falls	S29.39729; E027.39298
Makhaleng	MAK1	Qiloane Falls	S29.39734; E027.91447
Maletsunyane	JR9	Semonkong	S29.84736; E028.04906
Maletsunyane	JR8	Letlapeng Ha Phallang	S29.75510; E028.02410
Maletsunyane	DT6	Letlapeng Ha Phallang	S29.75240; E028.01550
Quthing	JR19	Letseng-la-Letsie	S30.34880; E028.15750

2.2 Eastern ESU Survey sites

The Eastern ESU survey was undertaken from 7 August to 14 August 2017. A total of 18 sites were surveyed (Table 2). The location of the sites was identical to those surveyed by Skelton et al (2001) with the exception of the Mothae River sites which were identical to those surveyed by Paxton (2011).

(See Appendix A for maps of each river with sampling sites; photos of all sampling sites captured in database to be shared with LHDA).

Table 2: Survey sites for the Eastern ESU, August 2017.

EASTERN ESU			
River	**Site**	**Location**	**GPS Coordinates**
Tsoelikane	TSO1	Sehlabathebe National Park	S29.88475; E029.11990
Tsoelikane	JR18	Sehlabathebe National Park	S29.89750; E029.12050
Mangaung	JR14	Ha Mamokae	S29.53110; E029.25940
Sani	JR14B	Ha Mamokae	S29.55620; E029.24250
Sani	DT11	Sani Flats	S29.56320; E029.27210
Mothae	MOT1A	Mothae Mine	S28.94530; E028.79540
Mothae	MOT2A	Mothae Mine	S28.95700; E028.78790
Mothae	MOT2B	Mothae Mine	S28.95800; E028.78790
Mothae	MOT2C	Mothae Mine	S28.96090; E028.78940
Mothae	MOT2D	Mothae Mine	S28.96410; E028.78890
Mothae	MOT3A	Mothae Mine	S28.96770; E028.78680
Mothae	MOT3B	Mothae Mine	S28.97450; E028.78530
Mothae	MOT3C	Mothae Mine	S28.97800; E028.78190
Mothae	MOT3D	Mothae Mine	S28.98160; E028.77980
Matsoku	DT4	Mothae Mine	S29.01002; E028.76331
Matsoku	JR22	Ha Mpeli	S29.25930; E028.55830
Senqu	JR10	None	S28.92556; E029.02312
Moremoholo	JR11	None	S29.12486; E029.32668

2.3 Sampling Methods

All rivers were surveyed with a SAMUS SE 1000 backpack electrofisher, a 12V battery, trailing cathode and an anode attached to a hand-held net. Electrofishing was conducted using the zigzag, single-pass method (Bateman et al., 2005). Fish were kept alive in a bucket filled with river water, identified to species level, counted and measured for fork length (FL mm) and then released (Figure 4). Water quality parameters (water temperature (° C), dissolved oxygen (mg. L^{-1}), electrical conductivity (EC) (µS.cm^{-1}) and pH were recorded at three random points at each site. (Note: The only exception was for the Mothae River sites which were typically >250m long. In this case, a minimum of five water quality readings were taken) (Figure 5).

Each sampling site was classified using the procedure outlined in Shelton et al. (2016) where the proportional composition of four river biotopes was visually assessed at each site. The biotopes include "slow-shallow", "slow-deep", "fast-shallow", and "fast-deep". Areas with depths <50cm were classified as shallow and areas with depths >50cm were classified as deep. Areas where the surface was smooth with minimal flow were classified as slow and areas where the surface was rippled or broken were classified as fast.

Mean channel width (m) was calculated from three random width measurements at each site. Mean depth (cm) was estimated from ten measurements at random points in the channel. The only exception was for the Mothae River sites which were typically >250m long. In this case, a minimum of 10 width measurements and 20 depth measurements were recorded (Figure 5).

Figure 4: A – Connecting the SAMUS 1000 E Electrofisher; B – Electrofishing on the Bokong River; C – *P. quathlambae* kept alive in buckets; D – *P. quathlambae* measurement

Figure 5: Measurements of channel width and water quality were conducted at each site

2.4 Data Analyses

The presence/absence data for fish at each sampling site were compared with previous surveys (Steyn et al., 1996; Skelton et al., 2001; Paxton, 2011; Shelton et al., 2016), where specific sampling site data were available.

Fish density (fish/100m^2) was computed for each species using the approach described by Rall (1999) where the efficacy of the electrofishing gear (or probability of capture) is assumed to be 60% (based on the average conductivity of rivers in the Lesotho Highlands) such that:

Density (fish/100m^2) = ((∑Catch/E)/river area sampled) x 100

where *E = 60% = 0.6;* and

river area sampled = mean width (m) x total length (m)

Mean density estimates were compared to those from 1995 (Steyn et al., 1996), 2001/2001 (Skelton et al., 2001), 2006 (Rall and Sephaka, 2008) and 2013 (Shelton et al., 2016). Abundance at specific sampling sites was compared with results from Steyn et al (1996) and Shelton et al (2016).

Catch per unit effort (fish/hr) was computed for each species using the approach described by Paxton (2011) where:

CPUE (fish/hr) = ∑Catch/Effort; and

effort = time fished (hours)

CPUE was only estimated for the Mothae River such that the methods were aligned with those from the Paxton (2011) survey.

Population estimates for *P. quathlambae* were calculated as:

Population size = ((∑Catch/Efficiency)/river area sampled) x (Length x Width) where

Length = distance (km) from lower distribution limit (e.g. waterfall) to point where river gradient is 1:40 and steeper; and

Width = average width of river over its length (km^2)

Population size estimates were compared to those obtained by Skelton et al (2001).

Length frequency distributions (FL mm) were computed for each species.

Habitat data (channel width (m), depth (cm), temperature (°C), dissolved oxygen (mg. L^{-1}) and conductivity (µS. cm^{-1})) from August 1995 (Steyn et al., 1996) and August 2017 (present survey) were log-transformed and one-way PERMANOVA was conducted with the fixed factor “Year” in order to determine if there were any significant differences.

For rivers where no previous habitat data were available, data were log-transformed and one-way PERMANOVA was conducted with the fixed factor “River” in order to determine if there were any significant differences in habitat and water quality conditions. PERMANOVA was conducted in R using the ‘vegan’ and ‘adonis’ packages.

3. Results

3.1 Mohale ESU – Native Range

No fish were recorded at any of the sampling sites in the Bokong- or Jorodane River. The presence/absence of fish from surveys conducted in 1995, 2003, 2006, 2013 and 2017 is shown in Table 3. (See Appendix B for presence/absence of fish at different sampling sites in different survey years).

Table 3: Presence/absence of *P. quathlambae* and *L. aeneus* in the Senqunyane-, Bokong- and Jorodane River from surveys conducted in 1995 (Steyn et al., 1996), 2006 (Rall and Sephaka, 2008), 2013 (Shelton et al., 2016), and 2017. “+” = present; “- “= absent

		Survey Year			
River	**Species**	**1995**	**2006**	**2013**	**2017**
Senqunyane	*P. quathlambae*	+	+	-	-
	L. aeneus	-	-	+	+
Bokong	*P. quathlambae*	+	+	+	-
	L. aeneus	-	-	+	-
Jorodane	*P. quathlambae*	+	+	+	-
	L. aeneus	-	-	+	-

Only *P. quathlambae* was recorded in the 1995 and 2006 surveys of the Senqunyane-, Bokong- and Jorodane River, while *L. aeneus* was the only species recorded during the 2013 and 2017 surveys of the Senqunyane River and, in August 2017, no fish were recorded in the Bokong- nor Jorodane River (and Senqunyane River – Site S34).

Habitat and water quality data from August 1995 and August 2017 are shown in Table 4. There was no significant difference in habitat and water quality conditions between August 1995 and August 2017 for the Senqunyane (Pr > F = 0.668), Bokong (Pr > F = 0.1) nor Jorodane (Pr > F = 0.3) River (Table 5).

Table 4: Comparison of habitat and environmental data from surveys conducted in August 1995 (Steyn et al., 1996) and August 2017 (present study) in the Senqunyane- (Sites marked “S”), Bokong- (Sites marked “B”) and Jorodane- (sites marked “J”) River. Note that only those parameters and sampling sites for which there were a full complement of data were included. “P” = pool; “F” = flat; “G” = glide; “SS” = slow shallow: “SD” = slow deep; “FS” = fast shallow: “FD” = fast deep.

		Channel Morphometry		Biotope comp (%)				Water quality			
Site	**Year**	**Width (m)**	**Depth (cm)**	**SS**	**SD**	**FS**	**FD**	**Temp (° C)**	**DO (mg. L^{-1})**	**Cond (µS. cm^{-1})**	**pH**
S24	**1995**	7	30			P		8	10	100	7
	2017	12	50	40	50	10	0	-	-	-	-
S25	**1995**	7	35-50			P		14	10	100	7
	2017	13	25	75	5	20	0	14	10	53	8
S28	**1995**	18	200			P		16	10	80	7

Site	Year	Channel Morphometry		Biotope comp (%)				Water quality			
		Width (m)	Depth (cm)	SS	SD	FS	FD	Temp (° C)	DO ($mg.L^{-1}$)	Cond ($\mu S.cm^{-1}$)	pH
	2017	22	0.40	65	30	5	0	9	8	43	8
S29	**1995**	9	23			F		8	11	50	7
	2017	14	60	30	70	0	0	15	10	59	8
S34	**1995**	7	32.5			P		8	11.2	40	-
	2017	9	52	45	50	5	0	11	8	32	7
B4	**1995**	5	20			F&G		12.6	9	50	6.6
	2017	11	26.5	60	0	40	0	11	8	46	7
B6	**1995**	5	40			P		13	9.5	60	6.5
	2017	7	28.3	50	0	50	0	11	9	43	8
B8	**1995**	5	30			F&P		6	12	100	6.6
	2017	7	38.9	60	30	10	0	13	8	43	7
J6	**1995**	9	20			G		14	9.2	70	7.8
	2017	12	44.6	25	25	50	0	10	9	69	7
J7	**1995**	6	32.5			F&P		14	9.2	70	7.8
	2017	15	33.90	60	5	25	10	9	8	64	7
J8	**1995**	7	27.5			G		10.2	8.7	60	6.7
	2017	9	32.20	85	0	10	5	1	11	49	7

Table 5: Test statistics from a one-way PERMANOVA with the fixed factor "Year" to analyse river-specific differences between habitat and water quality recorded in the Senqunyane-, Bokong-, and Jorodane River in August 1995 (Steyn et al., 1996) and August 2017. "Df" = degrees of freedom; "SumsofSqs" = sums of squares; "MeanSqs" = Mean of squares.

Senqunyane River						
	Df	**SumsofSqs**	**MeanSqs**	**F. Model**	**R^2**	**Pr > F**
Year	1	0.031308	0.031308	0.68268	0.10216	0.668
Residuals	6	0.275166	0.045861		0.89784	
Total	7	0.306474			1.00000	
Bokong River						
	Df	**SumsofSqs**	**MeanSqs**	**F. Model**	**R^2**	**Pr > F**
Year	1	0.032135	0.032135	2.8422	0.41539	0.1
Residuals	4	0.045225	0.011306		0.58461	
Total	5	0.077360			1.00000	
Jorodane River						
	Df	**SumsofSqs**	**MeanSqs**	**F. Model**	**R^2**	**Pr > F**
Year	1	0.016069	0.016069	1.9957	0.33285	0.3
Residuals	4	0.032207	0.0080518		0.66715	
Total	5	0.048276			1.00000	

3.2 Mohale ESU – Translocated Range

P. quathlambae were recorded in the Jorodane-, (n = 38), Makhaleng- (n = 11), and Maletsunyane River (n = 41) (Table 6). No fish were recorded in the Quthing River. Mean density and population estimates are shown in Table 7 and Table 8, respectively.

Table 6: Presence/absence of *P. quathlambae* in the Jorodane-, Makhaleng-, Maletsunyane- and Quthing River from surveys conducted in 2006 (Rall and Sephaka, 2008) and 2017.

		Survey Year	
River	**Species**	**2006**	**2017**
Jorodane (above Pampiri Falls)	*P. quathlambae*	+	+
Makhaleng	*P. quathlambae*	+	+
Maletsunyane	*P. quathlambae*	+	+
Quthing	*P. quathlambae*	-	-

Table 7: Mean density (at 60% efficiency) of *P. quathlambae* (fish/100m2) in the Jorodane- (above Pampiri Falls), Makhaleng- and Maletsunyane River.

	Jorodane River	**Makhaleng River**	**Maletsunyane River**
Density of fish/100m^2 @ 60% efficiency	4.78	3.59	7.42

Table 8: Estimated population size of P. quathlambae in the Jorodane- (above Pampiri Falls), Makhaleng-, and Maletsunyane River.

	Jorodane River	**Makhaleng River**	**Maletsunyane River**
Reach length (km)	12.39	6.23	25.39
Average width (km)	0.009	0.007	0.010
Total area (km2)	0.112	0.044	0.254
Estimated population size (60% efficiency)	5 400	1 600	18 800

Mean density and estimated population size was highest for the Maletsunyane River (7.42 fish/100m^2; 18 800 fish) and lowest for the Makhaleng River (3.59 fish/100m^2; 1 600 fish).

Length frequency distributions are shown in Figure 6. Only adult fish (>5mm) were sampled in the Jorodane River. Similarly, the majority of fish (91%) sampled in the Makhaleng River were adults. A range of size classes were sampled in the Maletsunyane River where 31% of the fish (n = 13) were juveniles. In both the Jorodane- and Makhaleng River, juvenile *P. quathlambae* were observed but not captured.

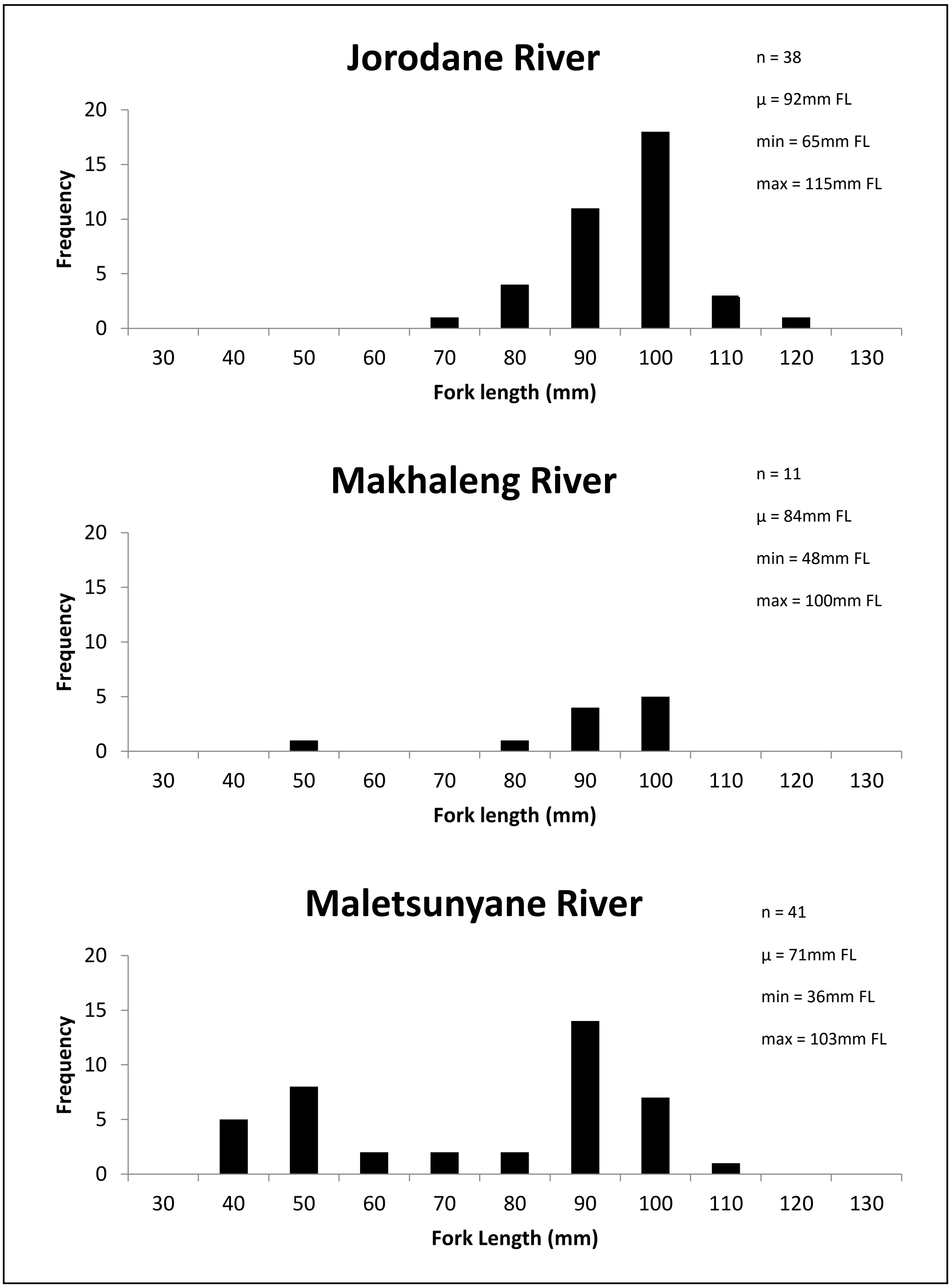

Figure 6: Length frequency distributions of *P. quathlambae* recorded in the Jorodane- (above Pampiri Falls), Makhaleng-, and Maletsunyane Rivers.

Figure 7: A = Adult P. *quathlambae*, Jorodane River; B = *adult P. quathlambae*, Makhaleng River; C = juvenile *P. quathlambae*, Maletsunyane River.

There was no significant difference ($p > 0.05 = 0.291$) in habitat and water quality parameters between the Jorodane-, Makhaleng-, Maletsunyane- and Quthing River (Table 9; Table 10).

Table 9: Habitat and water quality data recorded in the Jorodane-, Makhaleng-, Maletsunyane-, and Quthing River in August 2017.

River	**Width (m)**	**Depth (cm)**	**Temp (° C)**	**DO (mg/L)**	**Conductivity ($\mu S.\ cm^{-1}$)**	**pH**
Jorodane	8.95	30.03	9.27	9.62	56.95	7.35
Makhaleng	7.15	29.54	13.00	8.93	57.88	7.75
Maletsunyane	9.80	23.97	12.03	8.63	76.00	7.33
Quthing	8.51	30.03	7.93	9.37	73.47	7.02

Table 10: Test statistics from a one-way PERMANOVA with the fixed factor "River" to analyse differences in habitat and water quality recorded in the Jorodane-, Makhaleng-, Maletsunyane- and Quthing River in August 2017. "Df" = degrees of freedom; "SumsofSqs" = sums of squares; "MeanSqs" = Mean of squares.

	Df	**SumsofSqs**	**MeanSqs**	**F. Model**	**R^2**	**Pr > F**
"River"	3	0.0035807	0.00119357	1.3658	0.40578	0.291
Residuals	6	0.0052436	0.00087393		0.59422	
Total	9	0.0088243			1.00000	

3.3 Eastern ESU

P. quathlambae was recorded in the Tsoelikane River above (n = 9) and below (n = 6) the Tsoelikane Falls, in the Sani- (n = 2), Mothae- (n = 7), upper Matsoku- (Site DT4) (n = 33), Senqu- (n = 15) and Moremoholo River (n = 34) (Table 11). In contrast to 2000, no *P. quathlambae* were recorded in the Mangaung- or lower Matsoku River (Site JR22). No *O. mykiss* were recorded at any of the sites.

Table 11: Presence/absence of *P. quathlambae* and *O. mykiss* in the Tsoelikane-, Mangaung-, Sani-, Mothae-, Matsoku-, Senqu- and Moremoholo River from surveys conducted in 2000 (Skelton et al., 2001), 2011 (Paxton, 2011) and 2017. "+" = present; "-" = absent.

		Survey Year		
River	**Species**	**2000**	**2011**	**2017**
Tsoelikane (below falls)	*P. quathlambae*	+		+
	O. mykiss	+		-
Tsoelikane (above falls)	*P. quathlambae*	+		+
Mangaung	*P. quathlambae*	+		-
	O. mykiss	+		-
Sani	*P. quathlambae*	+		+
	O. mykiss	+		-
Mothae	*P. quathlambae*		+	+
Matsoku (upper)	*P. quathlambae*	+		+
Matsoku (lower)	*P. quathlambae*	+		-
Senqu	*P. quathlambae*	+		+
Moremoholo	*P. quathlambae*	+		+

Mean density and estimated population size increased in the Tsoelikane River (above falls) and the Moremoholo River and decreased in the Sani River and Senqu River. Estimated population size was highest in the Moremoholo (4 600 fish) and lowest in the Mothae (50 fish) (Table 12).

Table 12: Mean density of *P. quathlambae* (fish/100m^2) in the Tsoelikane-, Sani-, Mothae-, Matsoku-, Senqu-, and Moremoholo Rivers in 2000 (Skelton et al., 2001) and 2017 (present survey).

	Survey Year			
	2000		2017	
River	**Mean Density**	**Population (n)**	**Mean density**	**Population (n)**
Tsoelikane (below falls)	0.00	0	4.57	100
Tsoelikane (above falls)	4.30	2 100	7.49	3 600
Sani	3.00	600	0.43	100
Mothae	-	-	0.13	50
Matsoku (upper)	-	-	8.29	3 000
Matsoku (lower)	-	-	0.00	0
Senqu	4.10	600	2.10	320
Moremoholo	4.30	2 500	0.13	4 600

In the Mothae River, mean CPUE decreased from 12.9 fish/hr in 2011 to 1.6 fish/hr in 2017. CPUE from each site surveyed on the Mothae River in 2011 and 2017 is shown in Figure 7. No *P. quathlambae* were recorded at sites 2C, 2D, 3A, and 3B in 2017. At sites 3C and 3D, CPUE declined from 30.6 fish/hr and 21.9 fish/hr in 2011 to 9.6 fish/hr and 4.6 fish/hr in 2017, respectively.

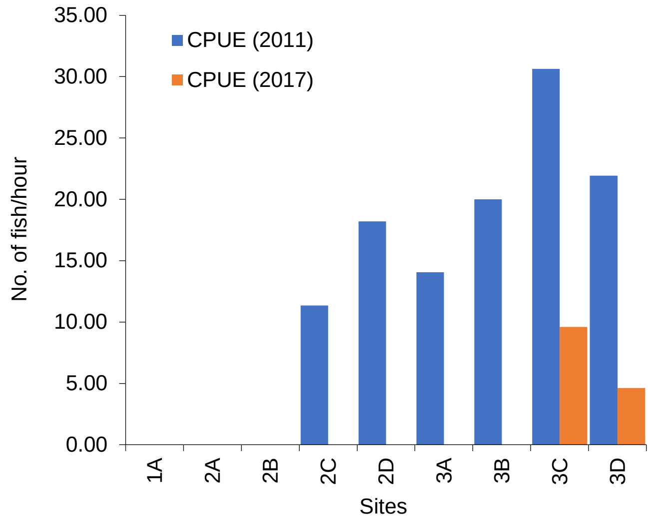

Figure 8: CPUE (fish/hr) of *P. quathlambae* from sampling sites on the Mothae River, April 2011 and August 2017.

Length frequency distributions are shown in Figure 9. Only adult fish (>50mm) were sampled in the Tsoelikane-, Sani-, Mothae- and Matsoku River (Figure 6A). Juveniles (20%; n = 3) and adults (80%; n = 12) were recorded in the Senqu River whereas the majority (94%; n = 31) of fish recorded from the Moremoholo River were juveniles. In the upper Matsoku River, juvenile *P. quathlambae* were observed but not captured.

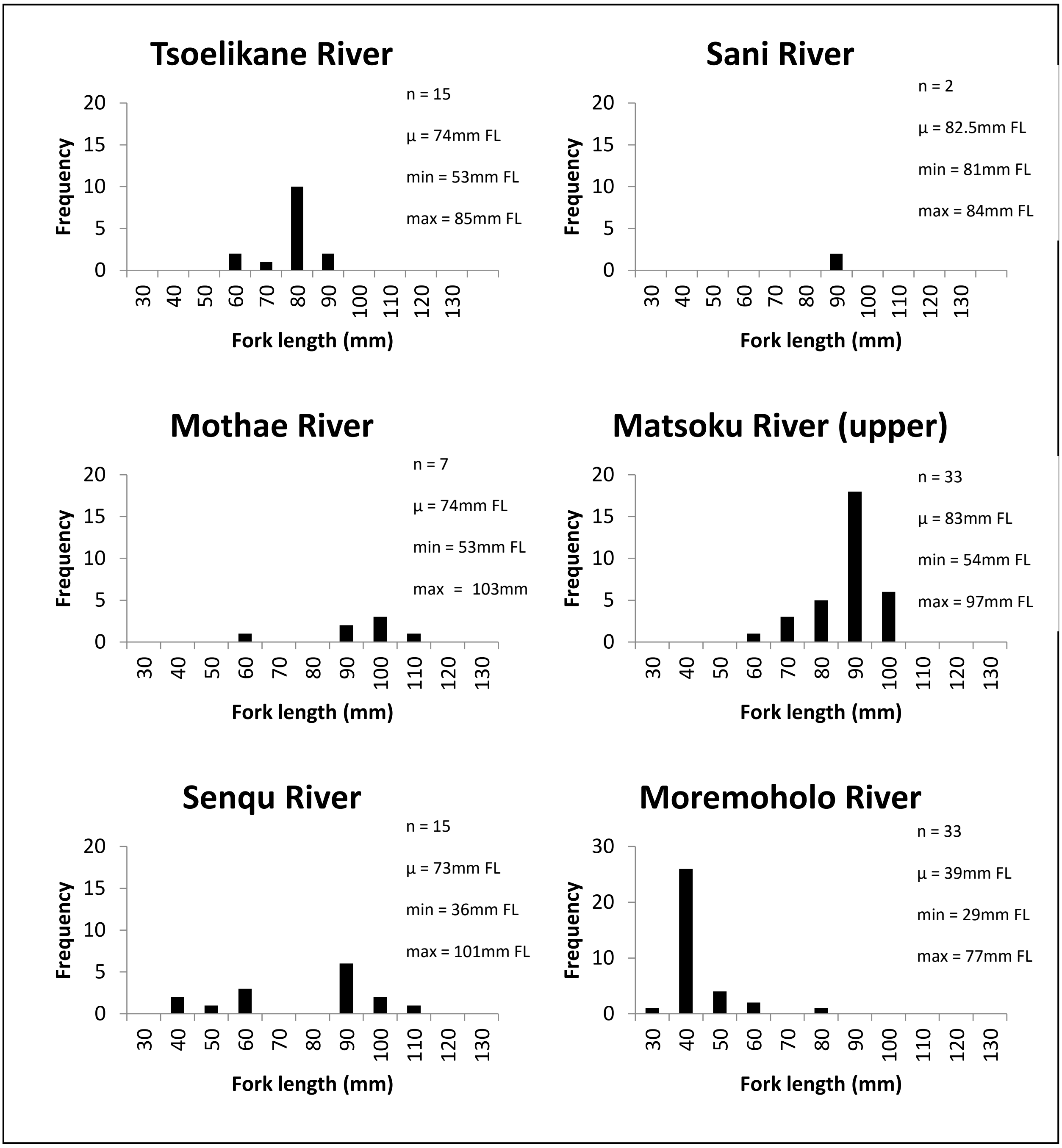

Figure 9: Length frequency distributions *of P. quathlambae* recorded in the Tsoelikane-, Sani-, Mothae-, Matsoku-, Senqu- and Moremoholo Rivers, August 2017.

Habitat and water quality data are shown in Table 13. The principal component analysis biplot (Figure 9) suggests that conductivity was higher in the lower Matsoku- and Mothae River whereas width and depth were higher in the Moremoholo-, Tsoelikane-, Senqu and upper Matsoku River. The results from the PERMANOVA analysis are presented in Table 14 and show that there was a significant difference (Pr > F = 0.025) in habitat conditions between rivers. The factor "River "explained 58% (R^2 = 0.5846) of the variability in the data.

Table 13: Habitat and water quality data recorded in the Tsoelikane-, Mangaung-, Sani-, Mothae-, Matsoku-, Senqu- and Moremoholo Rivers in August 2017.

River	Width (m)	Depth (cm)	Temp (° C)	DO (mg/L)	Conductivity (µS. cm^{-1})	pH
Tsoelikane	5.34	35.75	7.05	8.87	39.07	7.18
Mangaung	3.22	14.53	4.43	9.80	48.43	6.81
Sani	6.16	25.50	7.62	9.53	59.67	5.07
Mothae	3.23	22.02	7.18	8.97	62.84	6.22
Matsoku (upper)	9.22	40.60	4.63	10.27	53.53	7.02
Matsoku (lower)	7.23	51.89	10.97	7.67	220.73	4.64
Senqu	11.90	28.26	6.78	8.07	24.60	6.35
Moremoholo	17.17	34.25	13.43	6.93	44.23	2.82

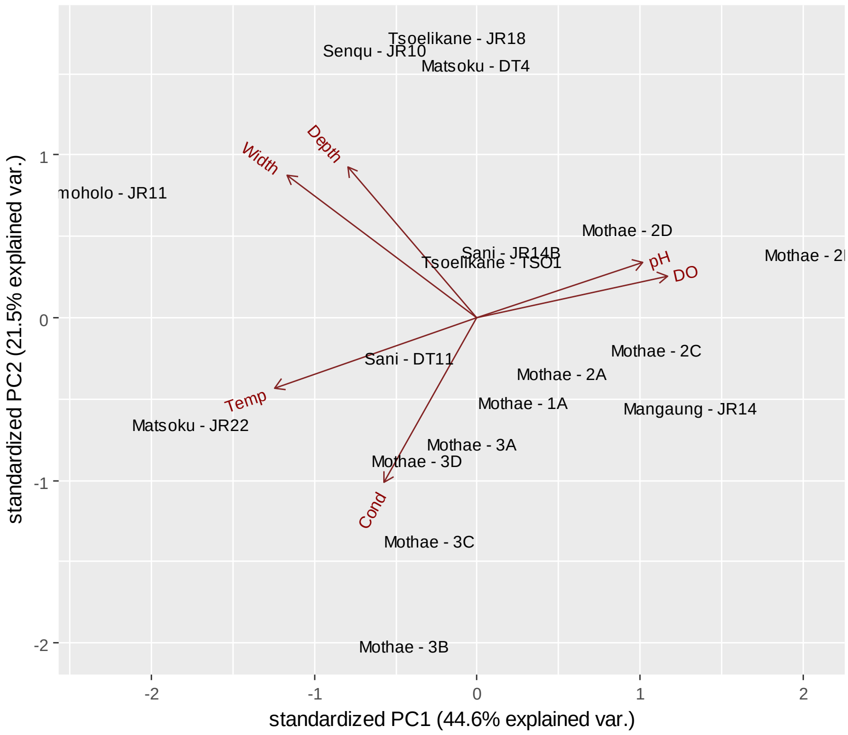

Figure 10: Principle component analysis biplot summarising habitat conditions in the Tsoelikane, Mangaung, Sani, Mothae, Matsoku (upper), Matsoku (lower), Senqu and Moremoholo Rivers, August 2017. PC 1 represents 44.6%, and PC 2 represents 21.5%, of the total variation in habitat conditions (based on the selected variables) among sites.

Table 14: Test statistics from a one-way PERMANOVA with the fixed factor "River" to analyse differences in habitat and water quality recorded in the Tsoelikane, Mangaung, Sani, Mothae, Matsoku (upper), Matsoku (lower), Senqu and Moremoholo River in August 2017.

	Df	SumsofSqs	MeanSqs	F. Model	R^2	Pr > F
"River"	6	0.047303	0.0078838	2.5801	0.5846	0.025
Residuals	11	0.033612	0.0030556		0.4154	
Total	17	0.080914			1.00000	

4. Discussion

4.1. Mohale ESU – Native Range

The results from the May 2017 survey (McCafferty et al. 2017) and this survey suggest that *P. quathlambae* Mohale ESU no longer occurs in its native range in the Senqunyane, Bokong- and Jorodane Rivers. The reason for the disappearance of the minnow is most likely a result of predation and competition from the non-native *L. aeneus* in these rivers (Shelton et al., 2016). This hypothesis is supported by an analysis of habitat data from August 1995 and August 2017 that shows no significant difference in habitat conditions in any of the rivers. Although these habitat data are by no means exhaustive, there was no apparent land use activity at the survey sites that would result in riparian habitat degradation severe enough to contribute to the disappearance of *P. quathlambae*.

The absence of *L. aeneus* in the Bokong- and Jorodane River in August 2017 is probably a result of seasonal migrations back into Mohale Dam during the winter months, behaviour that has been observed at both Mohale Dam (Tseliso Mothakathi, personal communication, May 2017) and Katse Dam (Nthimo, 2000). It is possible that some *L. aeneus* may occupy large, deep pools during the winter months (Figure 10) as is the case on the Bokong River flowing in to Katse Dam (Ntate Lebina, personal communication, May 2017). This has yet to be confirmed in any of the rivers of the Mohale Catchment. Nevertheless, it appears that annual upstream migrations of *L. aeneus* from Mohale Dam during the summer months have led to the disappearance of *P. quathlambae* Mohale ESU throughout its native range.

4.2. Mohale ESU – Translocated Range

The translocated populations of *P. quathlambae* in the Jorodane-, Makhaleng-, and Maletsunyane River have persisted and the translocation of *P. quathlambae* into the Quthing River has probably failed. These results corroborate those of Rall and Sephaka (2008).

No data are available with which to compare the mean density of the translocated populations from this survey and previous surveys. However, mean densities are all higher than those recorded in the Jorodane River (2.52 fish/100m^2) in 1999 (Rall, 1999), and the Bokong- (1.63 fish/100m^2) and Senqunyane River (1.31 fish/100m^2) in 2008. While potentially encouraging, these estimates should be treated with caution until such time as follow-up surveys have been conducted that account for seasonal variation and encompass longer reaches of river with additional sampling sites. The length frequency data suggest that the Maletsunyane River population is healthy with several size classes including juveniles, sub-adults and adults. Only 1 juvenile fish was captured in the Makhaleng and none were caught in the Jorodane, although they were seen in both rivers. The same population size structure (100% adult fish) was observed in the Jorodane River in 2005 and Rall et al., 2005 expressed concern about the survival of this population. However, a wide range of length classes (25mm – 105mm) were recorded in the follow up survey in 2008 (Rall and Sephaka, 2008). Similarly, in the Tsoelikane River, Rondorf (1975) sampled only adults (79mm – 115mm; μ = 100mm) in 1974-1975 whereas a "wide range" of size classes, including small juvenile fish, were sampled in 1988 (Cambray and Meyer, 1988) suggesting an improved size structure in that year compared to 1974-1975. The size classes recorded in this survey may not adequately represent the size structure, and health, of the population. It would be imprudent to draw any conclusions on the health of the translocated populations until such time as follow-up surveys have been conducted in different seasons and covering a more extensive area. There may be significant variability in spawning success and recruitment in these systems which can only be investigated through ongoing monitoring.

Research into assessing the genetic identity of the translocated populations and their resemblance to the parent stocks should be considered. Populations established by translocation often exhibit reduced genetic variability (Stockwell et al, 1996; Stockwell and Leberg, 2002) and impacts can include loss of fitness and increased extinction rates (Markert et al., 2010). Typically, determining genetic identity should occur after five or more hydrological cycles to allow for a period of colonisation and to establish a pattern of gene flow (Rall et al., 2005). As the translocations occurred in 2002/2003, a study of the genetics of these populations would contribute to understanding the success of the translocations and what implications this may have for the continued survival of the Mohale ESU.

Although detailed habitat quality assessments were outside the ambit of this survey, the Quthing River catchment was heavily overgrazed with widespread loss of grass cover and invasion by Karroid bush species, specifically the woody *Chrysocorma* spp. This is typical of mismanaged and heavily degraded mountain grassland habitats in the Drakensberg (Acocks, 1988). Furthermore, erosion was widespread and the river banks were slumping in many areas (Figure 10). While habitat and water quality conditions were not significantly different from the other rivers surveyed, it is possible that failure of *P. quathlambae* to establish itself in the Quthing River is due to habitat degradation in the catchment. Unfortunately, there is no habitat data available from previous years. Nevertheless, the survey results cannot completely exclude the possibility that *P. quathlambae* may persist in other areas.

Figure 11:River bank slumping, erosion and extensive invasion of *Chrysocorma* spp in the Quthing River catchment.

4.3. Eastern ESU

P. quathlambae were present in all of the river systems sampled by Skelton et al (2001) and Paxton (2011) with the exception of the Mangaung River, a small tributary of the Sani River. An unexpected (and encouraging) result was the presence of *P. quathlambae* (n = 6; mean density = 2.74 fish/100m^2) in the Tsoelikane River below the Tsoelikane Falls (Figure 11). In 1970, *P. quathlambae* occurred in "abundant numbers" below the falls whereas trout were few and far between as a result of "unfavourable environmental conditions for this species" (Pike and Tedder, 1973; Skelton et al., 2001). However, the "exact opposite" was recorded in 2000 – trout had become abundant and it was concluded that the population of *P. quathlambae* below the falls was extinct (Skelton et al., 2001). Our survey shows that this is not the case. It is possible that environmental variability significantly influences the abundance of *O. mykiss* to the extent that *P. quathlambae* can persist in the system by capitalising on periods during which conditions are unfavourable for *O. mykiss*, and predation and competition are reduced. In a study conducted in a number of New Zealand streams, low flow conditions prevented non-native brown trout *Salmo trutta* from eliminating native galaxiid (Galaxius spp.) populations. The authors concluded that, as galaxiids evolved in these systems, they were more resilient than brown trout to the stress imposed by low flow conditions (Leprieur et al., 2006). Ellender and Weyl (2015) investigated the response of the Eastern Cape redfin (*Pseudobarbus afer*) to a major flooding event and recorded no significant difference in the occurrence of juveniles or adults before and after the flood. The resilience of *P. afer* to significant hydrological disturbance was attributed to its evolution in an environmentally stochastic river system. Without detailed hydrological or habitat data, we cannot speculate as to the extent of disturbance in the Tsoelikane River and whether or not this is responsible for the fluctuating abundance of *P. quathlambae* and *O. mykiss*. However, the ability of *P. quathlambae* to persist in the Tsoelikane River may be a result of its resilience to stochastic abiotic events, such as drought or flooding, and the susceptibility of non-native *O. mykiss* to such events.

Figure 12: P. quathlambae sampled below the Tsoelikane Falls

Skelton et al. (2001) regarded the "small" Sani River population as the most vulnerable Eastern ESU population due to the threat posed by rainbow trout. While trout were only present in "low numbers" at the time (2000 survey), it was predicted that the onset of favourable conditions for trout would eventually lead to the extinction of *P. quathlambae* in this system. In August 2017, only two *P. quathlambae* and no trout were recorded. Mean density and estimated population size of *P. quathlambae* decreased from 4.1 fish/100m^2 and 600 fish in 2001 to 0.43 fish/100m2 and 100 fish in 2017, respectively. The Sani River catchment area was visibly degraded with the most obvious impacts being overgrazing, widespread erosion and invasive vegetation, river bank slumping, litter and the construction of a tar road and bridge across the river. Despite this, the absence of *O. mykiss* in the Sani River was unexpected. Discussions with the chief at Ha Mamokae revealed that during the construction of the road two years previously the river had been heavily fished by construction workers with gillnets, seine nets, and hook-and-lines. He had not seen any trout since. Management at Sani Mountain Lodge also informed us that they no longer offered trout fishing in the Sani River due to "a lack of fish" (Chantel Spargo, personal communication, August 2017). The removal of seemingly significant numbers of *O. mykiss* is a positive development for the survival of *P. quathlambae* in the Sani River (Skelton et al., 2001). However, the low numbers of *P. quathlambae* recorded, even after the decline of trout, may indicate that habitat degradation is a major threat to the continued survival of this population.

No fish were recorded in the Mangaung River (a tributary of the Sani River). The Mangaung River was the most shallow (mean depth = 14.53cm) and narrow (mean width = 3.22m) stream sampled during the survey. In 2000, "very low numbers" of *P. quathlambae* were recorded in the Mangaung River

(Skelton et al., 2001). It is possible that *P. quathlambae* move into the Mangaung River from the Sani River during summer for spawning as was hypothesised by Skelton et al (2001). This may explain the absence of fish from this river during the winter months although it may also be related to the low numbers recorded in the Sani River, and the impacts of habitat degradation.

Figure 13: The Sani River; note the erosion, river bank slumping and litter.

Numbers and CPUE of *P. quathlambae* in the Mothae River declined significantly since the 2011 survey. Unfortunately, there is no baseline habitat and water quality data for the Mothae River although its condition in 2011 was described as "pristine" (Bruce Paxton, personal communication, August 2017). This was not the case in 2017. Anthropogenic disturbances were very apparent and included the construction of a bridge across the river above Site 1A, where a road has been bulldozed across the river channel (Figure 14) and evidence of previous leakages from upstream mining activities (Palesa Monongoaha, personal communication, August 2017).

Water quality was characterised by typically higher conductivities (>80 µS. cm^{-1}) and highly variable pH, typically <5. Despite this, *P. quathlambae* were sampled albeit in far fewer numbers (n = 7) than in 2011 (n = 107). The decline may well be a result of anthropogenic habitat disturbance, however, it is recommended that a survey is conducted in summer to establish if *P.quathlambae* migrate downstream to the Matsoku River during the winter months.

Numbers and density of *P. quathlambae* at the upper Matsoku site were among the highest of all sites surveyed. Although only adults were recorded (n = 33; µ = 83mm FL; min = 54mm FL; max = 97mm FL), several juveniles were observed. It is unclear at this stage how far downstream and upstream this population extends. It is possible that *P. quathlambae* from the Mothae- and Matsoku Rivers move downstream into this area during winter before moving upstream again in summer for spawning purposes. The downstream distribution is probably controlled by increased anthropogenic disturbance as well as the impact of non-native fish that have moved into the catchment from Katse Dam via the Matsoku tunnel. Based on the survey of a downstream site (JR22), it would appear that there is little suitable habitat for *P. quathlambae* in the lower Matsoku River (see below).

Figure 14: The Mothae River; a road had been constructed across the river channel.

The site on the lower Matsoku River (JR22) was the most visibly degraded site (Figure 14). The site is located close to human settlements and agricultural activities. The upstream limit of the site is a crossing point for people and livestock, and probably serves as a washing location. Erosion and river bank slumping was extensive and a large amount of decomposing vegetation, alien vegetation, algae and litter was observed. Conductivity was far higher (approximately 220 µS. cm^{-1}) than any of the other sites surveyed. The LHDA BMU conducted a gillnet survey in October 2016 approximately 1km downstream of this site during which no fish were recorded despite reports of *L. aeneus* and possibly *O. mykiss* occurring in this stretch of river. It is possible that these species occur upstream of the weir above the site. However, the absence of *P. quathlambae* at the site was not unexpected and is probably a result of severe habitat degradation.

Figure 15: Sampling site on the lower Matsoku (JR22).

Results from the survey of the Senqu- and Moremoholo Rivers are largely consistent with those from 2000. In both surveys, the majority of fish recorded from the Moremoholo River were juveniles and the mean densities and estimated population sizes were higher in the Moremoholo River than in the Senqu River. The high proportion of juveniles at the Moremoholo site is probably a result of habitat at this site favouring younger age classes (Skelton et al., 2001) (Figure 15).

Skelton et al (2001) regarded the sites on the Senqu and Moremoholo as sanctuary areas, protected from trout by waterfalls and largely isolated from anthropogenic disturbances. Furthermore, the Phase II baseline fish survey concluded that the construction of Polihali Dam would have no direct impact on *P. quathlambae* populations above the falls as they are situated approximately 40km and 50km upstream of predicted FSL on the Senqu and Moremoholo, respectively (Bok, 2014). However, the construction of Polihali Dam is likely to significantly increase the density of yellowfish in these catchments and, as a result, increased numbers of yellowfish will undertake seasonal migrations up the Senqu- and Moremoholo River. Data from Mohale Dam show that yellowfish have migrated at least 30km upstream in the Senqunyane River and it possible that they move higher (McCafferty et al., 2017). Given the importance of these areas as sanctuaries, it is recommended that a comprehensive assessment of the waterfalls is conducted especially in light of the impacts that upstream migrations of significant numbers of yellowfish have had in the Mohale Dam catchment.

Figure 16: Sampling site on the Senqu River; juvenile *P. quathlambae* from the Moremoholo River.

5. Conclusions

1. *P. quathlambae* is no longer present in the Senqunyane-, Bokong- or Jorodane River (below Pampiri Falls). This is supported by both summer (2013) and winter (2017) electrofishing survey data.
2. The disappearance of *P. quathlambae* is most likely a result of predation by and possibly competition for habitat and food from *L. aeneus*.
3. The absence of *L. aeneus* from the Bokong River and Jorodane River during this survey is a result of seasonal migrations undertaken during winter downstream into Mohale Dam. It is possible that some *L. aeneus* may also occupy large, deep pools in these rivers during the winter months.

4. Translocated populations of *P. quathlambae* Mohale ESU were recorded in the Jorodane-, Makhaleng-, and Maletsunyane Rivers.
5. No fish were recorded in the Quthing River. The translocation of *P. quathlambae* Mohale ESU into the Quthing River has, in all likelihood, failed.
6. *P. quathlambae* Eastern ESU was recorded in the Tsoelikane-, Sani, Mothae-, Matsoku, Senqu and Moremoholo Rivers.
7. *P. quathlambae* Eastern ESU are not extinct in the Tsoelikane below the falls as previously suggested by Skelton et al (2001). This may be due to periods where environmental conditions do not favour *O. mykiss*, and predation of *P. quathlambae* is reduced.
8. Very few *P. quathlambae* (n=2) were recorded in the Sani River and no *O. mykiss* were present. Overgrazing and infrastructure development in the catchment have led to habitat degradation.
9. The Mothae River population is under threat from mining activities in the catchment. The low number (n = 7) of *P. quathlambae* recorded is either a result of habitat degradation and/or seasonal movement downstream into the Matsoku River.
10. It is unclear how large the population in the Matsoku River is. The upper Matsoku is relatively pristine in comparison to the highly degraded lower Matsoku. The extent of habitat degradation and occurrence of non-native fishes upstream from the lower Matsoku site is unknown at this stage.
11. The site on the Moremoholo River (JR11) appears to be a nursery area for *P. quathlambae*. Both the Senqu- and Moremoholo River can be regarded as sanctuaries for *P. quathlambae* as they are isolated from *O. mykiss* by waterfalls and the catchments are relatively healthy with little evidence of overgrazing.
12. The major threats to *P. quathlambae* Eastern ESU are non-native fish, specifically trout, and habitat degradation. The management units most at risk include: Sani River MU; Mothae River MU; Matsoku River MU

6. Recommendations

1. The construction of a barrier on the Senqunyane-, Bokong-, or Jorodane River to protect P. quathlambae is no longer necessary. Unless a programme focussing on the eradication of L. aeneus and restocking of P. quathlambae is initiated, construction of a barrier at this stage would serve no purpose. The feasibility of initiating such a programme is unclear at this stage.
2. Conservation planning and resource allocation for the Mohale ESU should now focus on the translocated populations in the Jorodane- (above Pampiri Falls), Makhaleng- and the Maletsunyane River.
3. The translocated populations are the last remaining remnants of P. quathlambae Mohale ESU. Follow-up surveys in early/late summer (October/November 2017 – March/April 2018 - before/after heavy rains and high flows) and the following winter (August 2018) are therefore strongly recommended. The surveys must encompass a number of sampling sites from the lower distribution limits (Pampiri Falls – Jorodane River; Qiloane Falls – Makhaleng River; barrier/weir bridge – Maletsunyane River) to the upper distribution limits where suitable habitat becomes limited (average gradient of 1:40 or more).
4. It is recommended that follow-up surveys of the Eastern ESU rivers are conducted in early summer. These surveys should encompass a number of additional sampling sites.
5. Baseline river health and rangeland assessments should be conducted simultaneously during these surveys.

6. The construction of Polihali Dam is likely to lead to high densities of yellowfish in the Senqu and Moremoholo River catchments. A comprehensive assessment of the suitability of the waterfalls in preventing upstream invasions of yellowfish is recommended.
7. On completion of the surveys, a comprehensive monitoring programme for the translocated and Eastern ESU populations must be designed. The programme needs to incorporate fish, river health and rangeland monitoring. The monitoring programme should be implemented after the completion of the follow-up surveys. It is recommended that monitoring is undertaken at least once every two years.
8. Consideration should be given to the following conservation measures:
    (1) Catchment restoration and management plans
    (2) Non-native fish eradication programmes (Eastern ESU)
    (3) Public participation, education and training

Finally, there is an urgent need to develop a strategic roadmap for the conservation of the translocated Mohale ESU and the Eastern ESU populations. Participants at a Workshop should include the LHWC, LHDA, Advance Africa and researchers who took part in the "Maloti Minnow Conservation Project" of 2000/2001. Conservation measures must be implemented to ensure the survival of one of Lesotho's flagship symbols of biodiversity, the endemic *thoboshana*.

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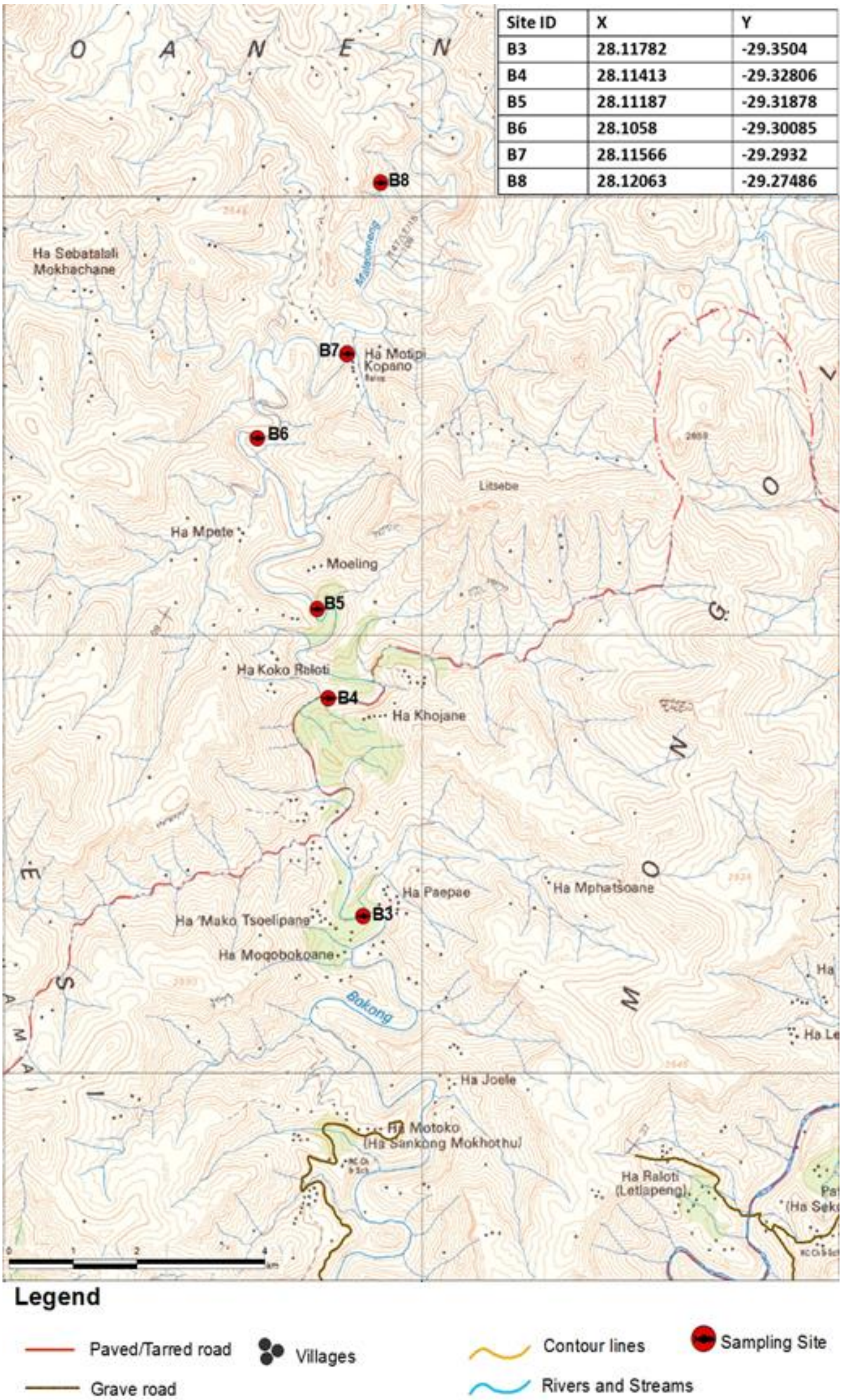
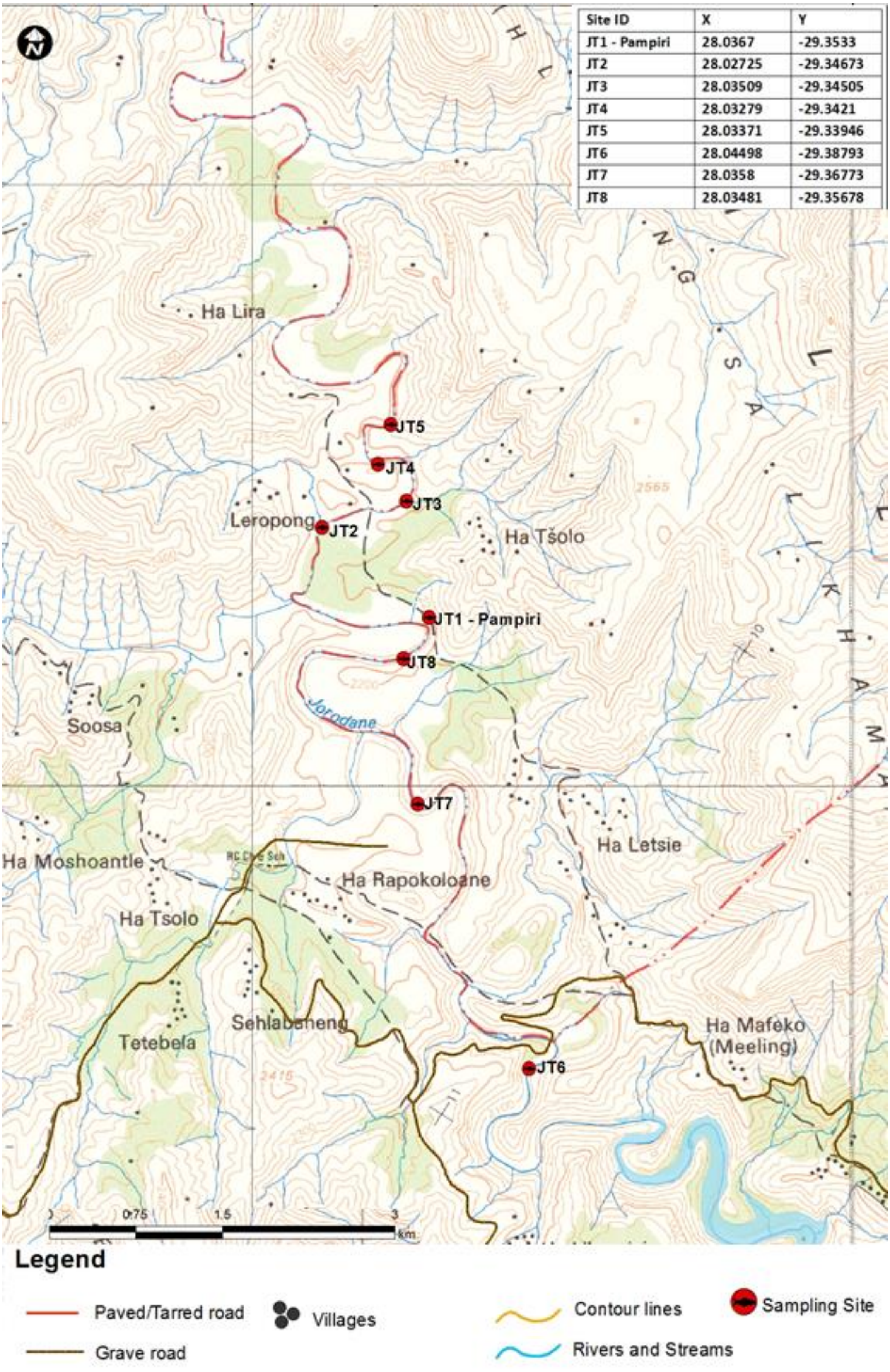
APPENDIX A

A.1. Bokong River

Site ID	X	Y
B3	28.11782	-29.3504
B4	28.11413	-29.32806
B5	28.11187	-29.31878
B6	28.1058	-29.30085
B7	28.11566	-29.2932
B8	28.12063	-29.27486

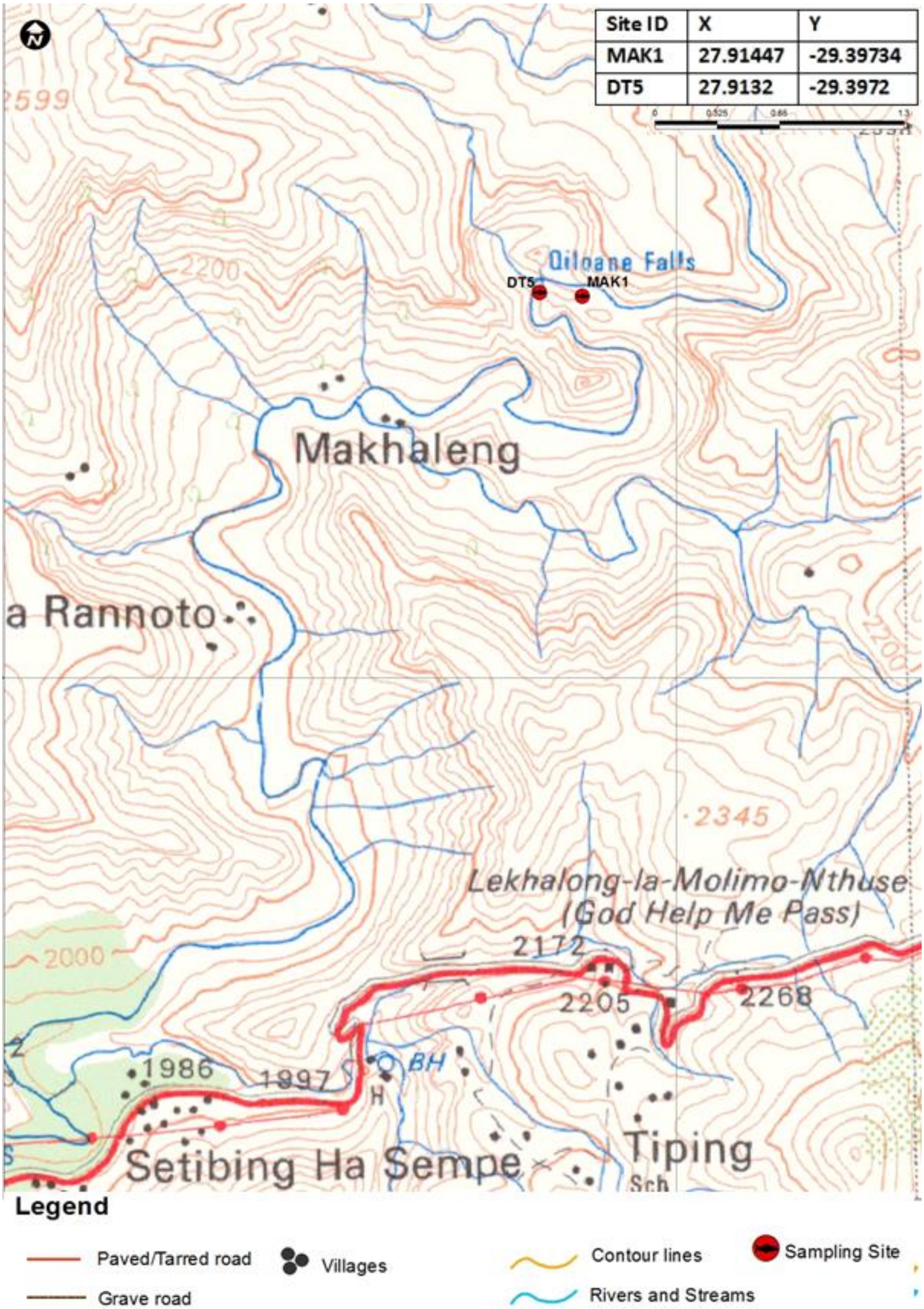


A.2. Jorodane River

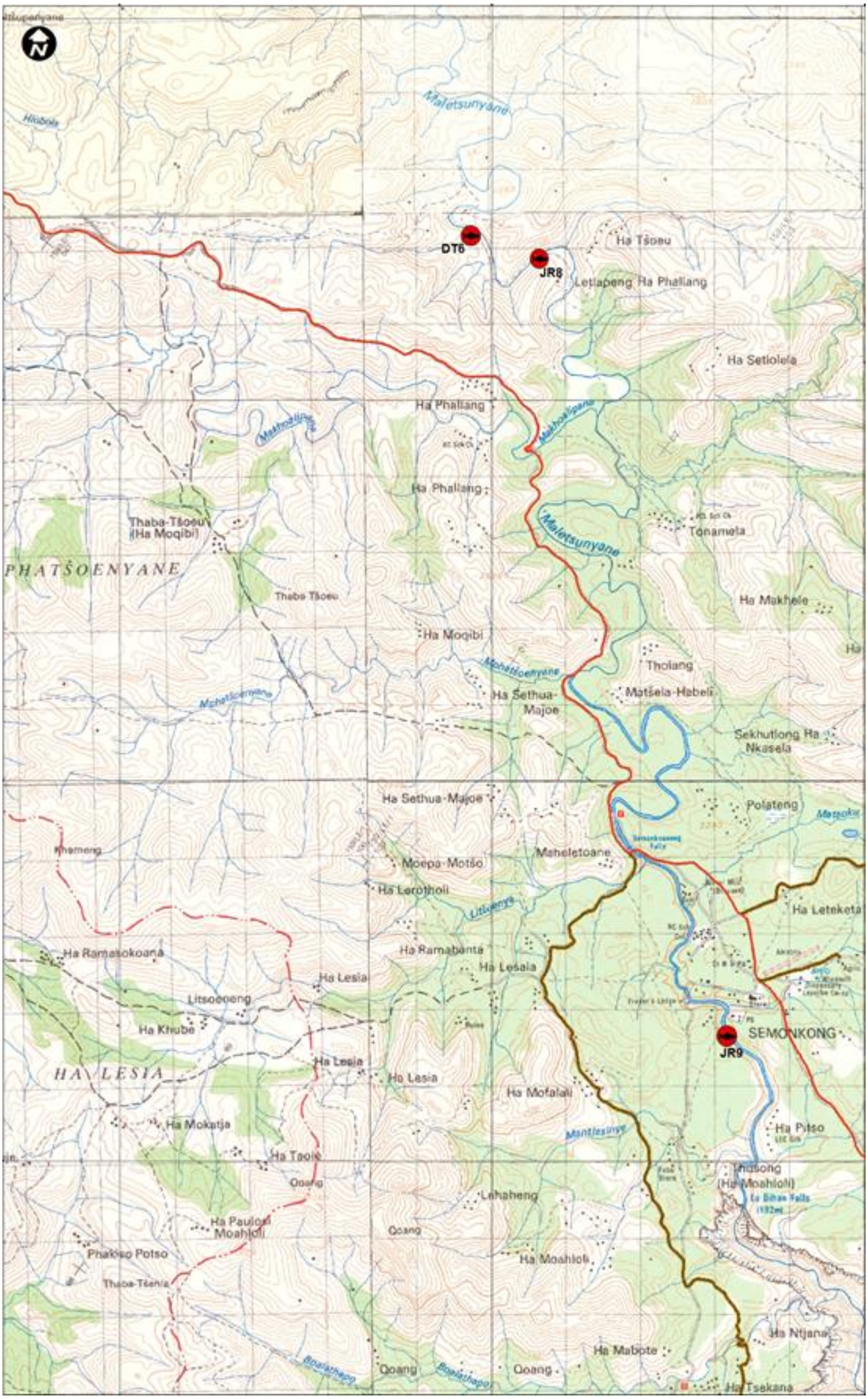


Site ID	X	Y
JT1 - Pampiri	28.0367	-29.3533
JT2	28.02725	-29.34673
JT3	28.03509	-29.34505
JT4	28.03279	-29.3421
JT5	28.03371	-29.33946
JT6	28.04498	-29.38793
JT7	28.0358	-29.36773
JT8	28.03481	-29.35678

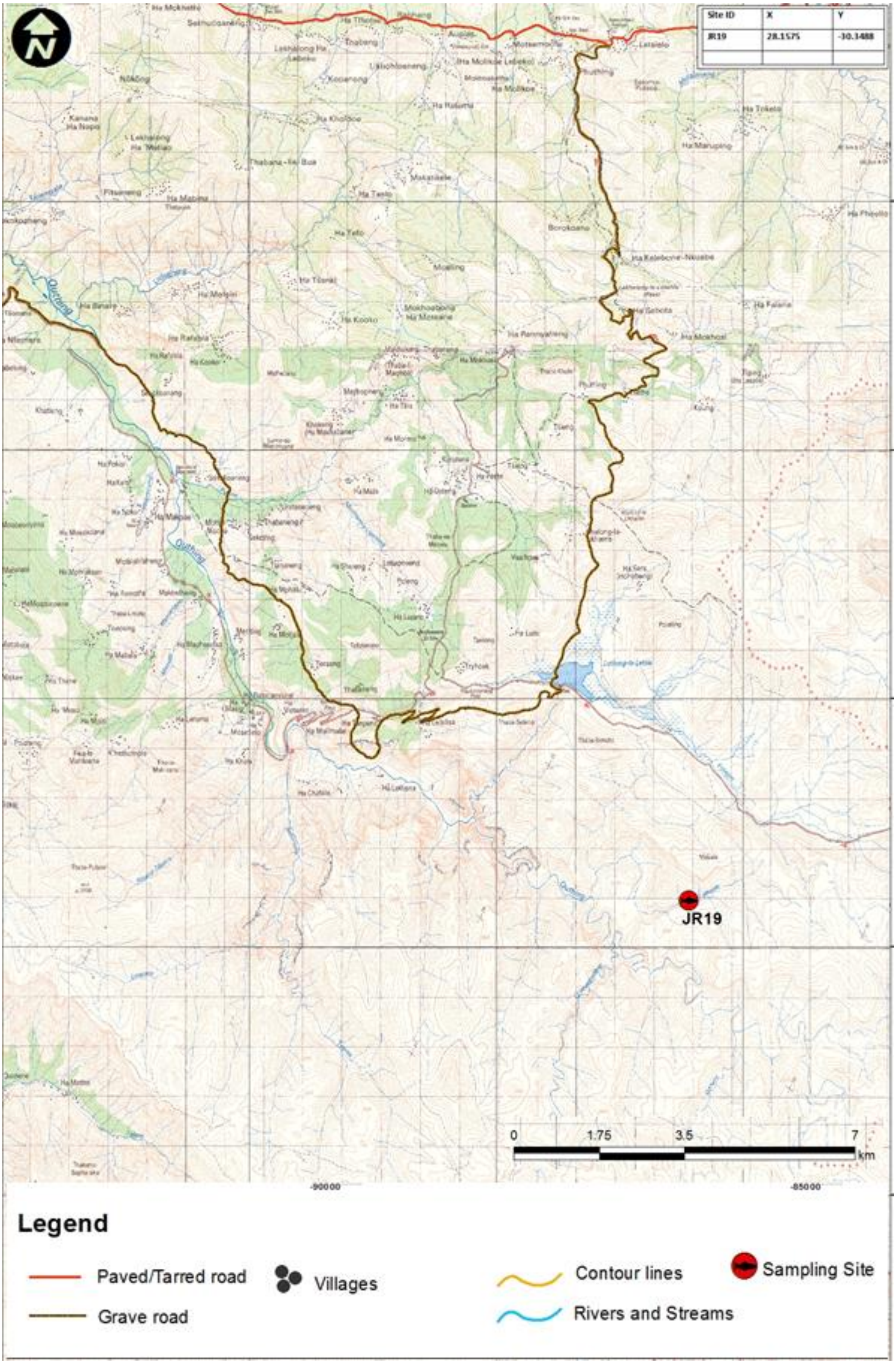
A.3. Makhaleng River



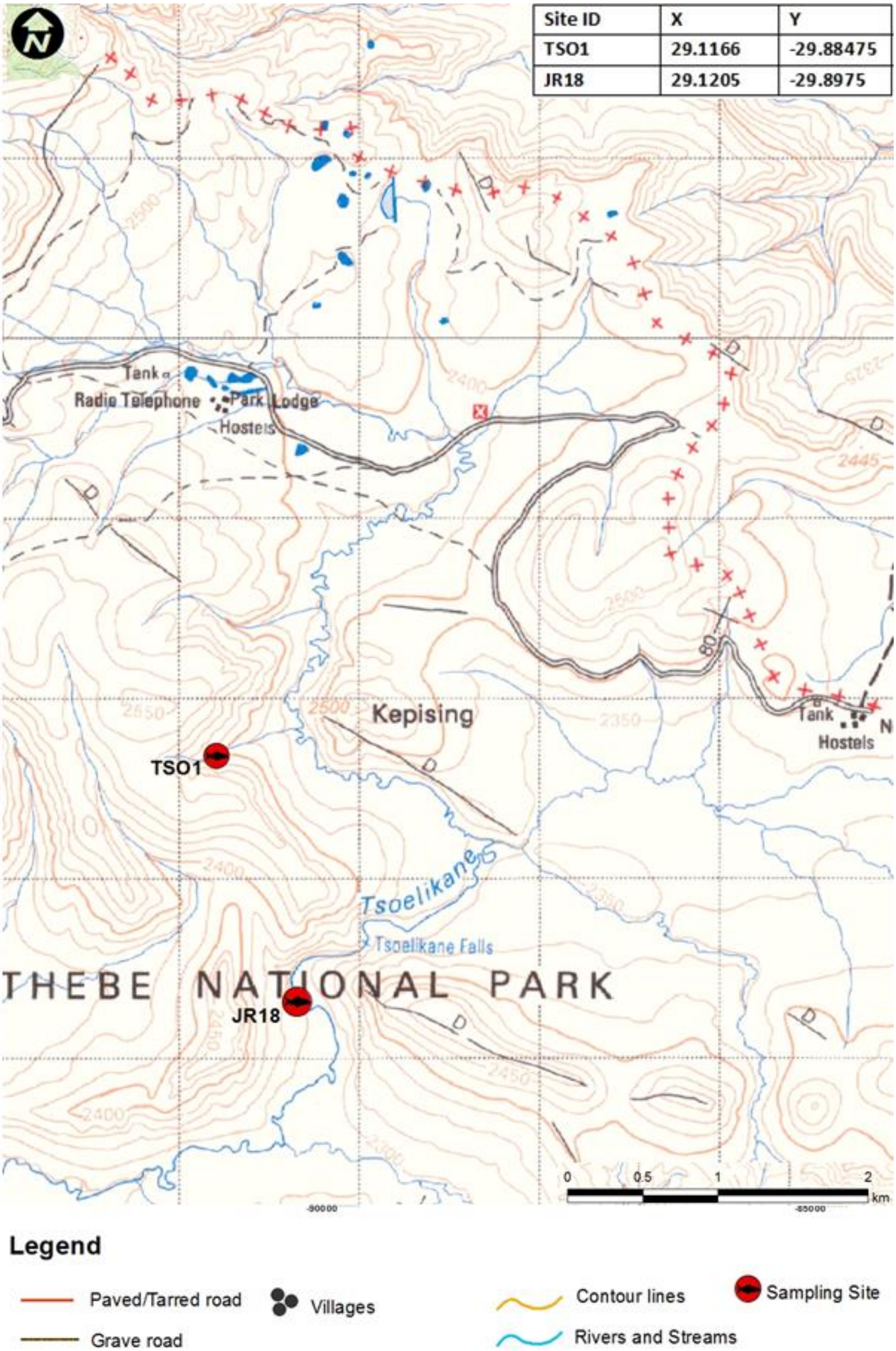
A.4. Maletsunyane River



A.5. Quthing River

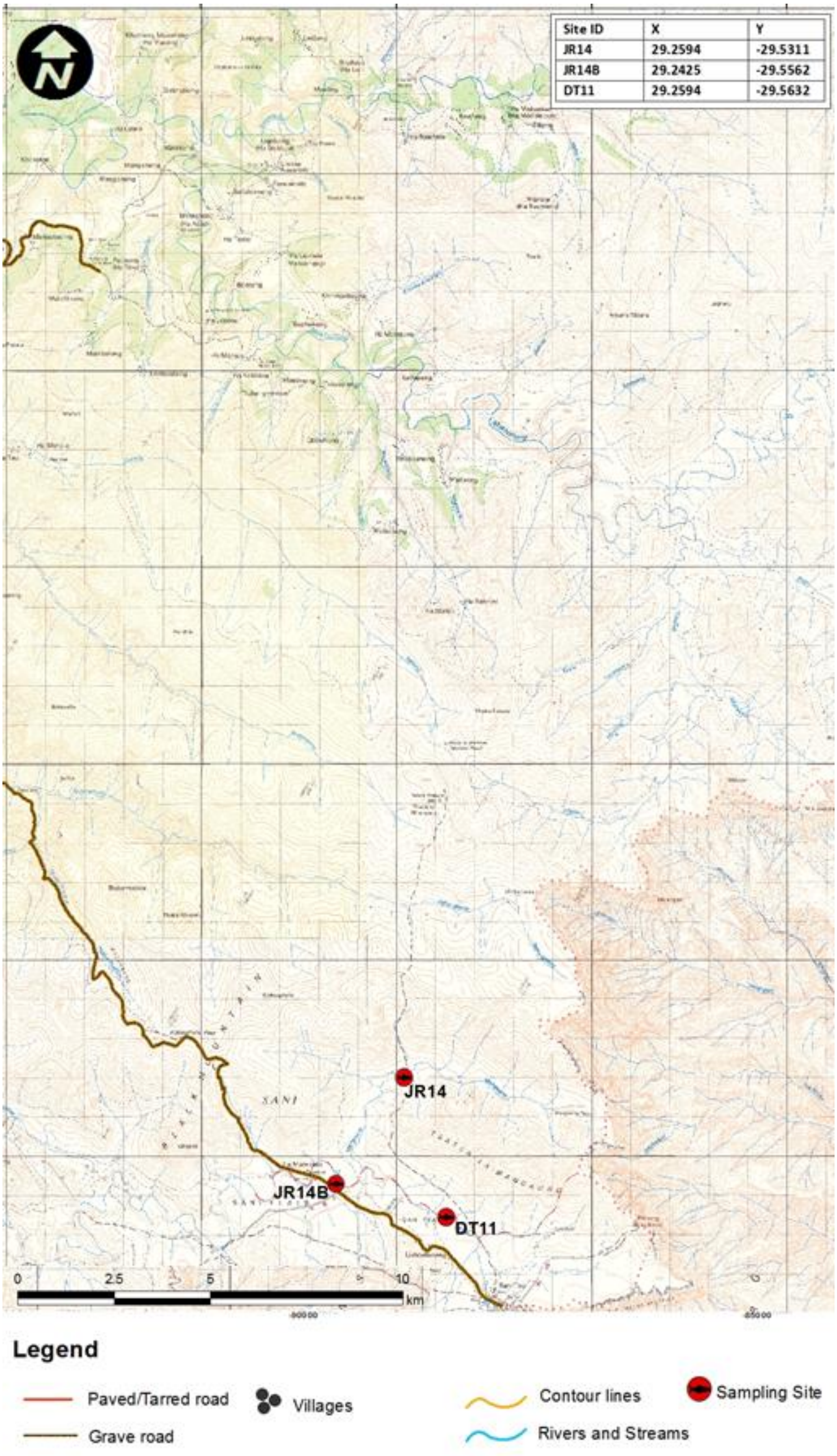


A.6. Tsoelikane River



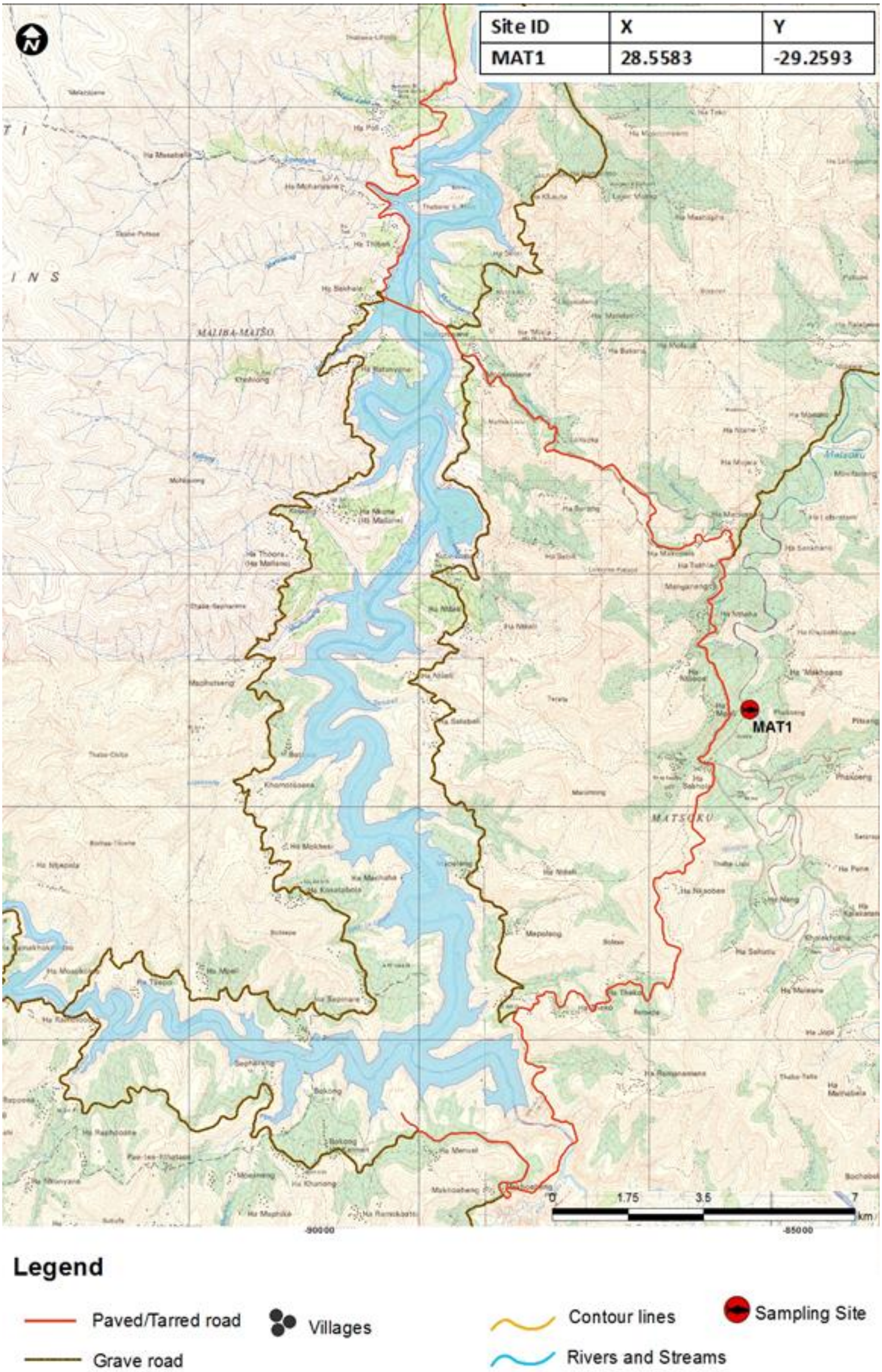
Site ID	X	Y
TSO1	29.1166	-29.88475
JR18	29.1205	-29.8975

A.7. Sani- and Mangaung River

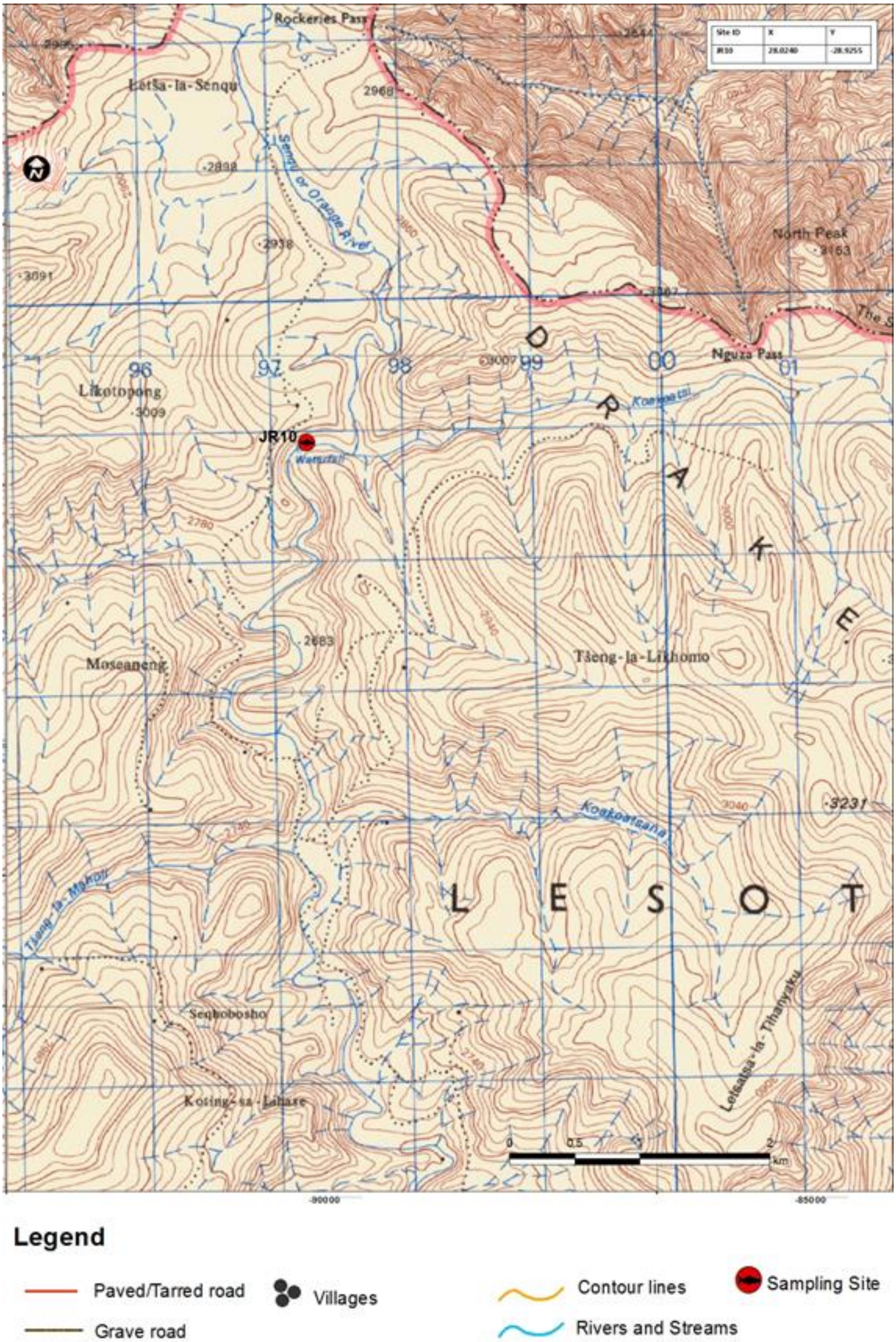


A.8. Mothae and upper Matsoku River

A.9. Lower Matsoku River

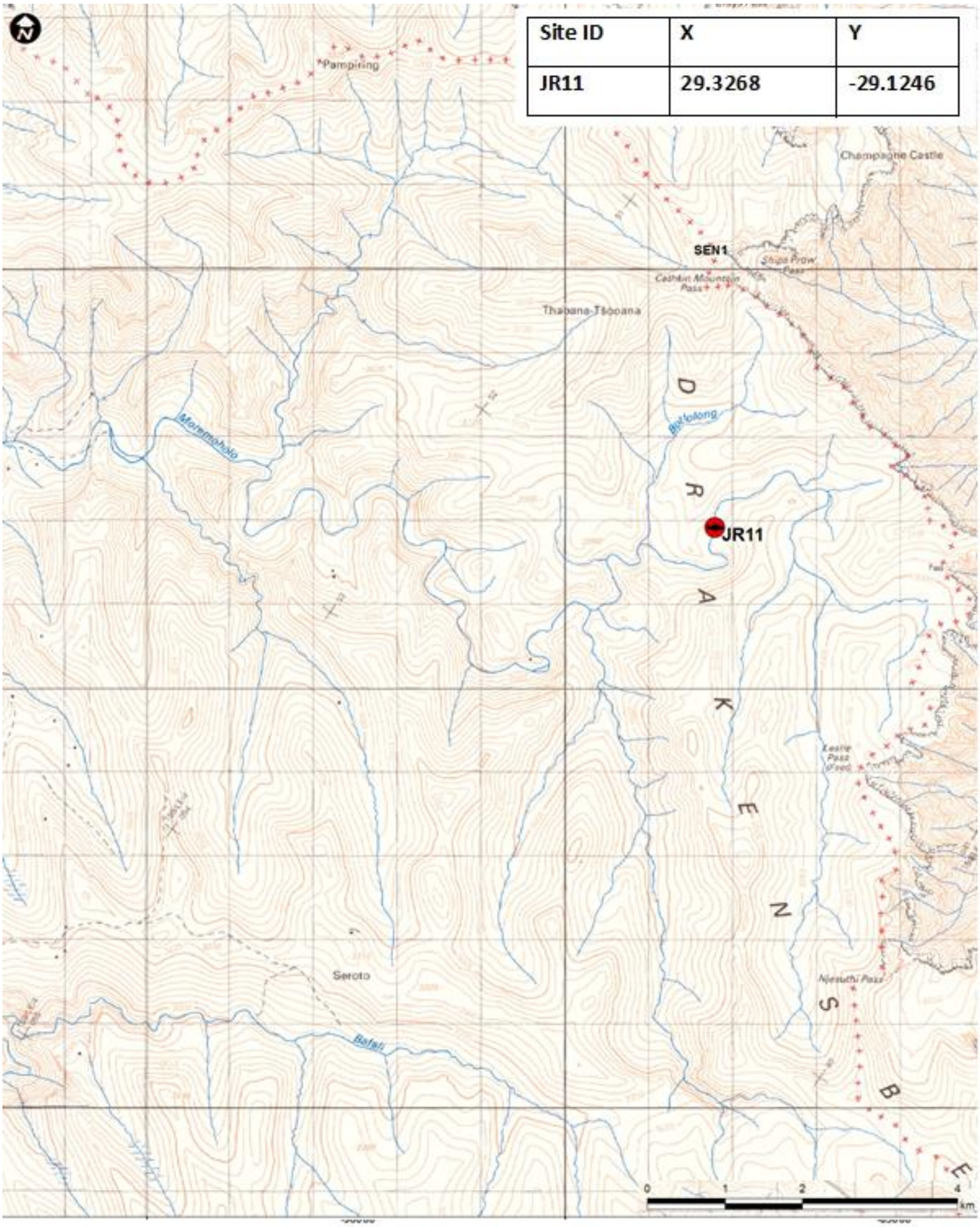


A.10. Senqu River



A.11. Moremoholo River

Site ID	X	Y
JR11	29.3268	-29.1246



Legend

Paved/Tarred road
Grave road
Villages
Contour lines
Rivers and Streams
Sampling Site

APPENDIX B

Table A1: Presence/absence of *P. quathlambae*, *L. aeneus*, and *L. capensis* at survey sites on the Senqunyane, Bokong, Jorodane River from surveys conducted in 1995, 2013, and 2017.

				Survey Year	
River	**Site**	**Species**	**1995**	**2013**	**2017**
Senqunyane	**S34**	*L. aeneus*	0	0	0
		L. capensis	0	0	0
		P. quathlambae	+	0	0
Senqunyane	**S29**	*L. aeneus*	0	+	+
		L. capensis	0	0	0
		P. quathlambae	+	0	0
Senqunyane	**S28**	*L. aeneus*	0	+	+
		L. capensis	0	0	0
		P. quathlambae	+	0	0
Senqunyane	**S25**	*L. aeneus*	0	+	+
		L. capensis	0	0	0
		P. quathlambae	+	0	0
Senqunyane	**S24**	*L. aeneus*	0	+	+
		L. capensis	0	0	0
		P. quathlambae	+	0	0
Senqunyane	**S22**	*L. aeneus*	0	+	+
		L. capensis	0	0	0
		P. quathlambae	+	0	0
Bokong	**B8**	*L. aeneus*	0	0	0
		L. capensis	0	0	0
		P. quathlambae	+	+	0
Bokong	**B7**	*L. aeneus*	0	0	0
		L. capensis	0	0	0
		P. quathlambae	+	+	0
Bokong	**B6**	*L. aeneus*	0	0	0
		L. capensis	0	0	0
		P. quathlambae	+	0	0
Bokong	**B5**	*L. aeneus*	0	+	0
		L. capensis	0	0	0
		P. quathlambae	+	+	0
Bokong	**B4**	*L. aeneus*	0	+	0
		L. capensis	0	0	0
		P. quathlambae	+	0	0
Bokong	**B3**	*L. aeneus*	0	+	0
		L. capensis	0	0	0
		P. quathlambae	+	0	0
Jorodane	**J8**	*L. aeneus*	0	0	0
		L. capensis	0	0	0
		P. quathlambae	+	+	0
Jorodane	**J7**	*L. aeneus*	0	0	0
		L. capensis	0	0	0
		P. quathlambae	+	+	0
		L. aeneus	0	+	0

Jorodane	**J6**	*L. capensis*	0	0	0
		P. quathlambae	+	0	0

Table A2: Presence/absence of *P. quathlambae* and *O. mykiss* at survey sites on the Tsoelikane, Mangaung, Sani, Mothae, Matsoku, Senqu and Moremoholo Rivers from surveys conducted in 2001, 2011 (Mothae) and 2017.

				Survey Year	
River	**Site**	**Species**	**2001**	**2011**	**2017**
Tsoelikane	**JR18**	*P. quathlambae*	0		+
		O. mykiss	+		0
Sani	**DT11**	*P. quathlambae*	+		0
		O. mykiss	+		0
Sani	**JR14B**	*P. quathlambae*	+		+
		O. mykiss	+		0
Mangaung	**JR14**	*P. quathlambae*	+		0
		O. mykiss	+		0
Mothae	**MOT1A**	*P. quathlambae*		0	0
		O. mykiss		0	0
Mothae	**MOT2A**	*P. quathlambae*		0	0
		O. mykiss		0	0
Mothae	**MOT2B**	*P. quathlambae*		0	0
		O. mykiss		0	0
Mothae	**MOT2C**	*P. quathlambae*		+	-
		O. mykiss		0	0
Mothae	**MOT2D**	*P. quathlambae*		+	-
		O. mykiss		0	0
Mothae	**MOT3A**	*P. quathlambae*		+	-
		O. mykiss		0	0
Mothae	**MOT3B**	*P. quathlambae*		+	-
		O. mykiss		0	0
Mothae	**MOT3B**	*P. quathlambae*		+	-
		O. mykiss		0	0
Mothae	**MOT3C**	*P. quathlambae*		+	+
		O. mykiss		0	0
Mothae	**MOT3D**	*P. quathlambae*		+	+
		O. mykiss		0	0
Matsoku	**DT4**	*P. quathlambae*		+	+
		O. mykiss		0	0
Matsoku	**JR21**	*P. quathlambae*		+	0
		O. mykiss		0	0
Senqu	**JR10**	*P. quathlambae*		+	+
		O. mykiss		0	0
Moremoholo	**JR11**	*P. quathlambae*		+	+
		O. mykiss		0	0