# Fish monitoring summary
# Meiringspoort diesel spill incident

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# 1. Introduction

Freshwater systems and their associated biota are among the most threatened ecosystems globally (Dudgeon et al 2006). Freshwater fishes represent an important component of freshwater ecosystems and are presently under threat from five major types of anthropogenic activity: habitat degradation and fragmentation; water use, including impoundment and abstraction; water quality deterioration; invasive alien species and overexploitation (Collares-Pereira and Cowx 2004; De Moor and Day 2013). Of these, the presence of non-native fish species is considered the greatest threat to native fishes of the Cape Fold Ecoregion (CFE), followed by the loss of habitat (Skelton 1983; Tweddle et al. 2009; Weyl et al. 2014). The CFE is one of the six aquatic ecoregions of Southern Africa and is characterised by an endemic and highly threatened freshwater fish fauna (Skelton 2001). Many indigenous fish species are range restricted and vulnerable to anthropogenic impacts such as water abstraction and pollution. One such example is the fish community of the Groot River that is located partially within the Swartberg Provincial Nature Reserve and forms part of the greater Gouritz River system. The Groot River is home to four native fish species and is listed as a nationally important fish sanctuary in the National Freshwater Ecosystem Priority Areas (NFEPA) Atlas. The native fish fauna of the Groot River consist of three cyprinid species, two of which are endemic to the Gouritz system, and a native anabantid that is endemic to the CFE (Figure 1). The endemic cyprinids are the smallscale redfin *Pseudobarbus asper* (Vulnerable) and the slender redfin *Pseudobarbus tenuis* (Near Threatened). The chubbyhead barb *Enteromius anoplus* (Least Concern) is not a Gouritz system endemic and has a wider distribution range to the north of South Africa. The Cape kurper *Sandelia capensis* (Data Deficient) is endemic to the CFE.

**Figure 1**: Smallscale redfin *Pseudobarbus asper* (Top-L), Slender redfin *Pseudobarbus tenuis* (Top-R), chubbyhead barb *Enteromius anoplus* (Bottom-L), Cape kurper *Sandelia capensis* (Bottom-R)

The motivation for the present survey was to investigate the short-term effects of a diesel spill into the Groot River that occurred on 15 August 2017. Based on a field report compiled shortly after the incident by Hilland Environmental, approximately 40 000L of diesel was spilled into the river following a truck accident towards the northern side of Meiringspoort. Spill containment measures were implemented by SpillTech and included the deployment of containment berms, removal of contaminated soils and the use of absorbent materials to soak up diesel for later removal. The use of BioSolve, a known hydrocarbon mitigation agent, was used to rehabilitate affected soils in the spill zone. It must be noted that due to the threatened nature of the native fish community, all fish collected from the impact zone was salvaged and relocated downstream from the lowest sampling sites and that the results of the surveys must be interpreted with this in mind.

## 2. Survey area and sampling methodology

The Groot River was sampled at nine sites within three zones in the river (Figure 2). These zones were a control zone (located upstream of the contaminated zone), an impact zone (between the spill site and the furthest downstream containment berm) and a downstream site located some distance downstream of the end of the impact zone (Table 1). Sampling was conducted for four sampling intervals: (1) two weeks after the spill - September 2017, (2) three months after - December 2017, (3) nine months after - June 2018 and (4) one year after - October 2018. In each zone, three replicate fyke net efforts were conducted to determine the fish community composition and to investigate whether there were differences between the three zones. Fyke nets were deployed for a soak time of 16 hours. All nets had a base width of 600mm and 4mm mesh size. All fish that were collected were measured and both fork length (FL) and total length (TL) was recorded, and catch per unit effort (CPUE) was calculated for each site. Habitat characteristics were noted for each site and water quality parameters (pH, temperature, conductivity and dissolved oxygen) were collected along with SASS5 sampling (reported separately).


**Figure 2**: Locality of the sampling sites within the control (C1-C3), impacted (I1-I3) and downstream (D1-D3) areas

**Table 1**: Geographical localities of sample sites

| Zone | Site number | DDS | DDE |
|---|---|---|---|
| | C1 | -32,527861 | 22,541667 |
| Control | C2 | -33,361806 | 22,541972 |
| | C3 | -33,362 | 22,542389 |
| | I1 | -33,372306 | 22,554083 |
| Impact | I2 | -33,372639 | 22,553889 |
| | I3 | -33,364194 | 22,558889 |
| | D1 | -33,409111 | 22,554361 |
| Downstream | D2 | -33,409056 | 22,555111 |
| | D3 | -33,409278 | 22,553583 |

# 3. Results of field surveys

## 3.1 Habitat assessments

### 3.1.1 Control zone

The sites in the control zone was located directly downstream of a causeway on the farm Middelwater towards the northern end of the Meiringspoort. For the initial September 2017 survey the river was flowing well and consisted of a number of medium sized pools (30-40 m long, max depth ±1.2m). The water was slightly turbid at the time of sampling. Pool substrate was mostly gravel, sand and mud that was covered in periphyton with some rocky riffle areas between. As with pool habitat, the majority of shallower habitat also had sand and mud substrate. Some of the pools also had some shale bedrock substrate but this was not common. Periphyton was present on all bedrock substrate. All pools had moderate amounts of undercut banks and root wads and marginal vegetation consisting of sedges. Woody debris was present in all pools. By the time of the December 2017 survey, the river had very little flow and the furthest upstream control site had dried up. Pool volume was severely reduced and only two sites were suitable for deploying nets. By June 2018 the entire control zone had dried up and no sampling could be conducted (Figure 3). The river was flowing again by October 2018 but pool volume was still reduced relative to the initial sampling event of September 2017.

### 3.1.2 Impact zone

Three sites were selected in the impacted zone, with two sites located near the upper end of the spill zone and the third site located at the end of the spill zone immediately upstream from the furthest downstream containment berm. For the September 2017 survey, the habitat at the first site was a small and relatively shallow pool (approximately 2m wide and less than 10m long) with a cobble substrate and abundant woody debris and marginal vegetation. In contrast, the other two netting areas were associated with large and deep pools in the river. These pools were characterised by a sand and mud substrate and the middle site (I2) had large shale bedrock banks on the left bank. Both sites had relatively turbid water and slow flow and marginal vegetation consisting of sedges. The drought effects observed at the control zone in December 2017 was also evident in the impact zone at this time as both the upstream and downstream sites were dry. Also, similar to the control zone, pool volume was reduced compared to the September 2017 sampling event. By June 2018 all three sampling sites within the impact zone had dried up and no sampling could be conducted (Figure 4). It must be noted that some water remained in the impacted zone close to sites I2 and I3, but these sites were not sampled due to very deep mud substrates which complicated setting and retrieving of nets. The river was flowing again by October 2018 but pool volume was still reduced relative to the initial sampling event of September 2017.

### 3.1.3 Downstream zone

The downstream sampling sites were located approximately 500m downstream of the rest stop at the Meiringspoort waterfall. The habitat in this zone was markedly different from the control and impacted zones in that it had more typical “mountain stream” type of characteristics. The substrate was mainly rock and cobble covered in periphyton with some larger boulders. Sand and gravel substrate was limited to pool areas. There was a roughly 1:1 mix of pool and run/riffle habitat and the water was far less turbid than in the control zone. Undercut banks and root wads were especially common in pool habitat. Brown algae was observed in shallow out of current areas. In contrast to the control and impacted sites, the river was flowing moderately well for all four sampling intervals and as a result all three sampling sites could be netted at each sampling interval.

**Figure 3:** Site 2 (C2) of the control zone during September 2017 and June 2018 indicating the impacts of severe drought and possible water abstraction.

**Figure 4:** Site 2 (I1) of the impact zone during September 2017 and June 2018 indicating the impacts of severe drought and possible water abstraction.

### 3.2 Fish survey results

#### 3.2.1 Control zone

For the September 2017 survey, the smallscale redfin *Pseudobarbus asper* was detected in two of the three netting efforts and the mean CPUE for the zone was 9.0 fish/effort (Figure 9). The size range of the redfins were 48-104 mm TL and a total of 27 fish were caught in this zone. The majority of the fish caught were either gravid females or males with tubercles and fish in the large size classes dominated the population (Figure 5a). The common platanna *Xenopus laevis* was also present (n=6). For the December 2017 survey size data provided evidence for a successful spawning season as the majority of fish caught were in the <40mm TL size class (Figure 5b). This was despite reduced flow and the fact that the furthest upstream sampling pool (C1) had dried up. The CPUE for the control zone for December was 57.0 fish/effort (Figure 9). Other biota caught included *X. laevis* adults (n=3) and tadpoles (n=48). Three adult frogs, provisionally identified as the Cape River frog *Amietia fuscigula* was observed along with two distinct types of tadpoles that were not *X. laevis* but could not be identified in field. The control zone was not sampled in June 2018 as all pools were dry. By October 2018, some flow was observed in the river but the pool volume was reduced compared to the September 2017 sampling. All pools were netted but only two fish were caught, resulting in a CPUE for the zone of 1.0 fish/effort. Other biota caught were 31 adult *X. laevis*, one adult *A. fusgicula* and numerous tadpoles of both species.

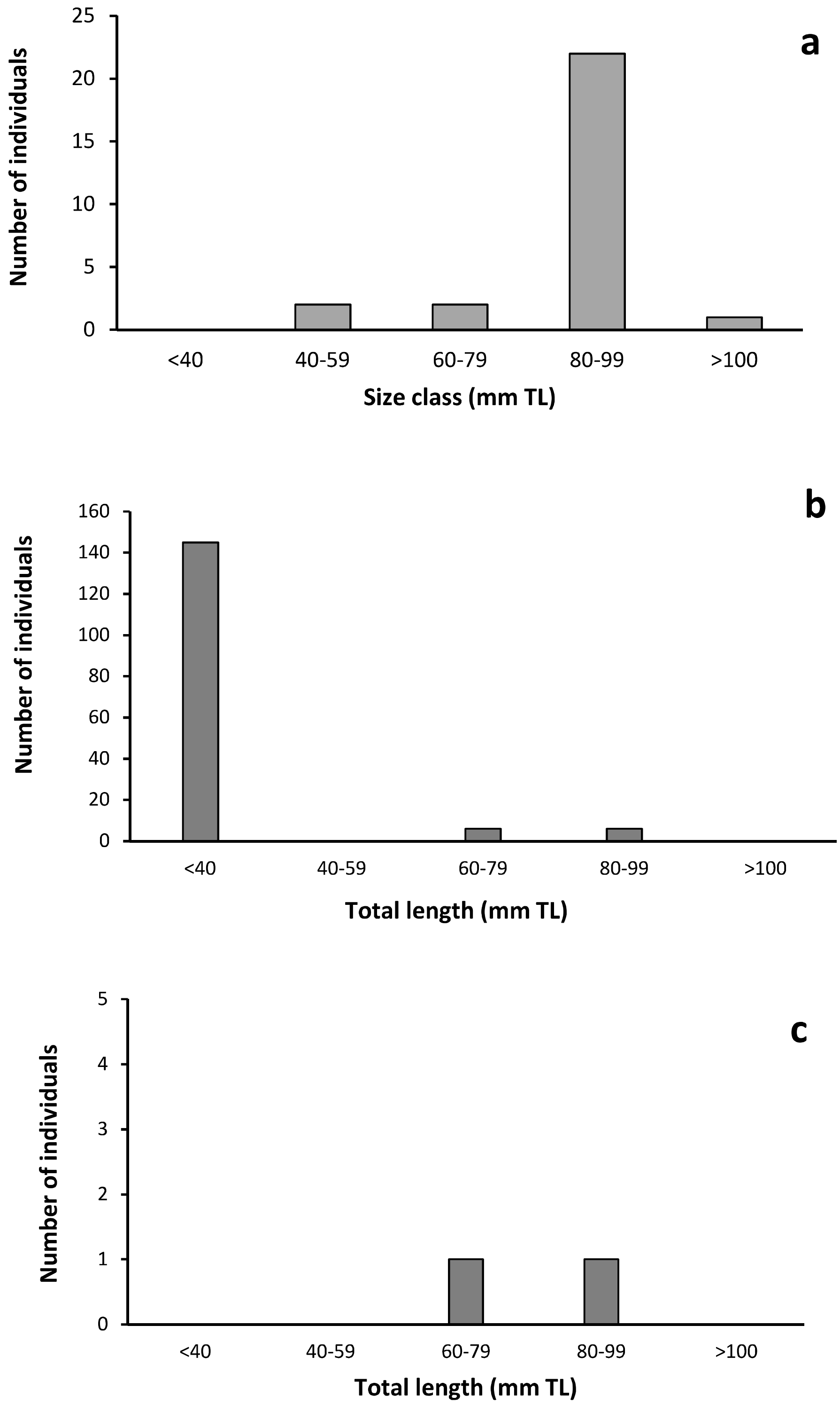



**Figure 5a-c**: Size class distribution of *P. asper* collected from the control zone of the Groot River during (a) September 2017, (b) December 2017 and (c) October 2018.

### 3.2.2 Impact zone

When inspecting the spill zone two days after the incident, several containment berms were observed in the affected zone and clean-up of contaminated soil was underway (Figure 6). The river was low at the time of the site visit with visible flow in riffle areas. It appeared that the spill had been contained by the furthest downstream berm (Figure 6). The SpillTech team reported that the area did receive rain but it appeared not to significantly affect the river's flow rate. No signs of affected animals were initially observed (with the exception of a dead duck and a dead frog), but dead and moribund *P. asper* were detected at approximately 14:00 on Friday 18 August in a small pool in the affected area. The fist dead fish were thus observed roughly four days after the spill.

**Figure 6**: Containment berms and clean-up of contaminated soil (left) and furthest downstream containment berm (right)

For the September 2017 survey, the smallscale redfin *Pseudobarbus asper* was detected in all three netting efforts and the mean CPUE for the zone was 22.3 fish/effort (Figure 9). The size range of the redfins were 36-96 mm TL and a total of 67 fish were caught in this zone. Very few fish in this zone were gravid females or males with tubercles and fish of all size classes were present. The most abundant size class were the 60-79mm TL class. (Figure 7a). There was large variation between the numbers of fish caught per sampling effort. The upper two sites yielded relatively low fish numbers (one and nine redfins respectively), while the net set at the end of the impacted zone yielded more fish (n=57) than all three control zone netting efforts combined. These results must however be interpreted with the differences in the habitat in mind. The common platanna *X. laevis* was also present (n=10) but no tadpoles were observed. All fish from the impacted zone were salvaged and moved to clean habitat at Spookdrif located at the entry to the Meiringspoort.

For the December 2017 survey, site I1 was dry and I3 was reduced in volume and yielded no fish. The middle site I2 was sampled and in addition to *P. asper* that was detected in the first survey, the slender redfin *P. tenuis* was detected, although in very low numbers (Figure 7b). For the June 2018 survey, all sample sites in the impacted zone had dried up and no fish were sampled during this survey. It must be noted however that some water remained in this zone in the form of a few isolated pools so any surviving fish would be able to find refuge in these pools. For the October 2018 survey, sites I1 and I2 were netted but I3 remained dry. No fish were detected at site I1 but 37 *P. asper* were caught at site I2, resulting in a CPUE value of 12.3 fish/effort for the zone. Fish ranged in size from 48-115mm TL and larger fish in the >80mm TL size class dominated the population (Figure 7c). More than 50% of individuals were observed to have well developed tubercles, indicating potential spawning activity.

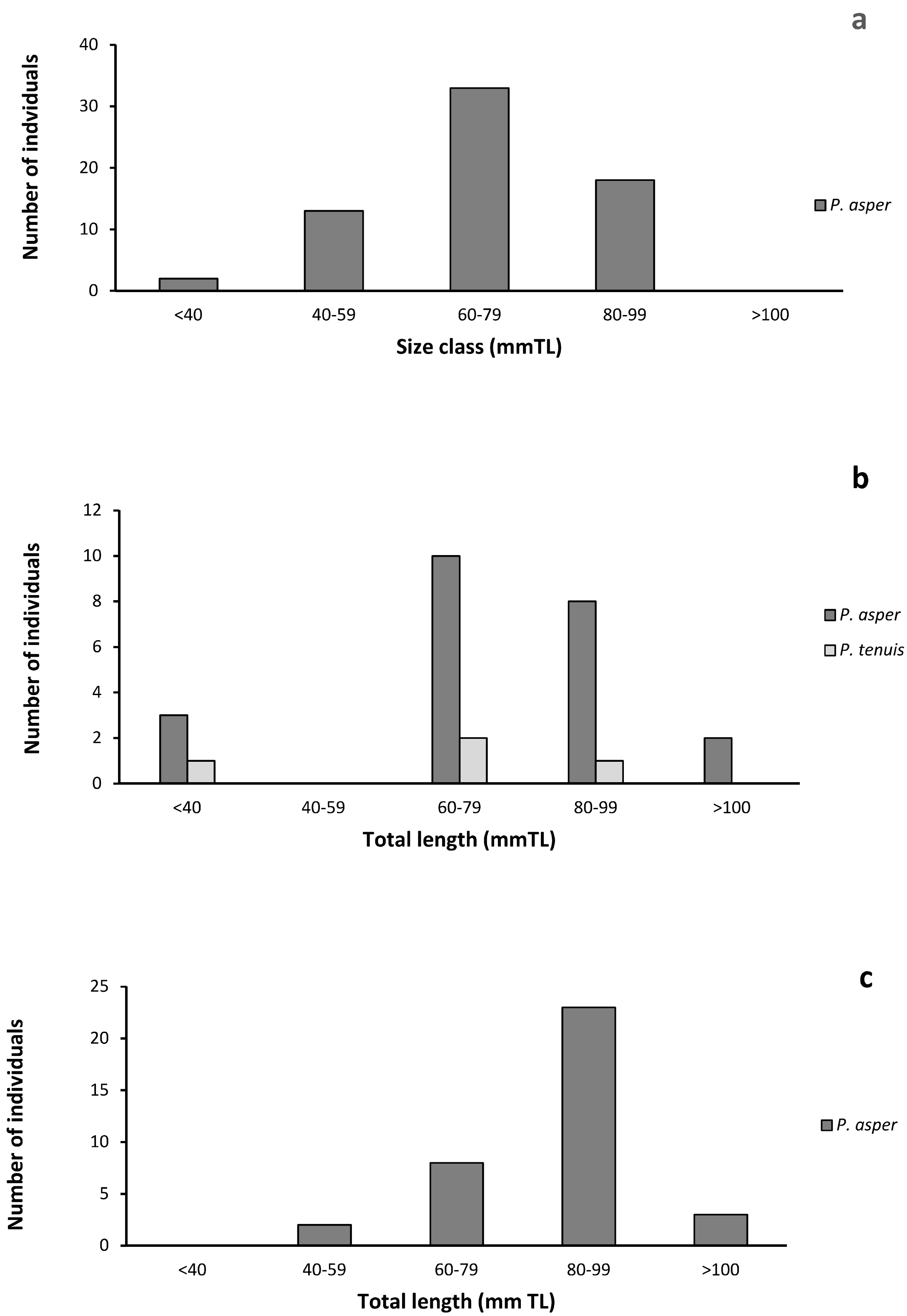
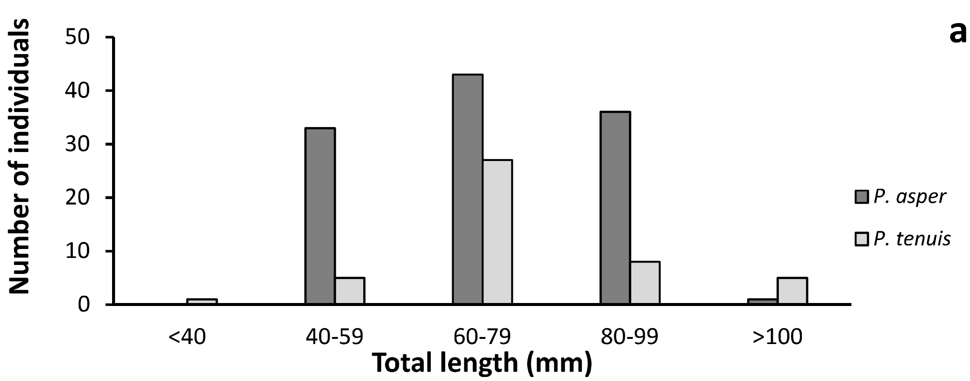



**Figure 7a-c**: Size class distribution of *P. asper* and *P. tenuis* collected from the impacted zone of the Groot River during (a) September 2017, (b) December 2017 and (c) October 2018.

### 3.2.3 Downstream zone

For the September 2017 survey, *P. asper* as well as the slender redfin *P. tenuis* was detected in all three of the netting efforts. A range of size classes were detected and the size range of the redfins were 44-102 mm TL for *P. asper* and 38-78 mm TL for *P. tenuis* (Figure 8a). A total of 160 fish were caught in this zone of which 47 (29%) were *P. tenuis* and the 113 (71%) were *P. asper*. The CPUE for the zone was 53.3 fish/effort (Figure 9). Some of the fish caught were either gravid females or males with tubercles but these were not common. The common platanna *X. laevis* was detected in low numbers and all size classes were observed. For the December 2017 survey, 152 fish were caught in four netting efforts, of which 44 were *P. asper* and 108 were *P. tenuis*. The mean CPUE for the sampling trip was 38.0 fish/effort (Figure 9). The size range of the redfins were 54-88 mm TL for *P. asper* and 40-90 mm TL for *P. tenuis*. There was limited evidence of successful spawning as there was few fish of less than 40mm TL detected during sampling (Figure 8b).

In contrast, for the June 2018 survey, young of year fish were abundant and the observed size range was 32-94 mm TL for *P. asper* and 20-88 mm TL for *P. tenuis* (Figure 8c). A total of 1378 *P. asper* were caught along with 306 *P. tenuis* resulting in a total of 1684 fish and a CPUE of 561.0 fish/effort (Figure 9). For the September 2018 survey, less fish were caught (n=916) of which there were 622 *P. asper* and 294 *P. tenuis*. This resulted in a CPUE of 318.0 fish/effort (Figure 9). The size range of the redfins were 32-104 mm TL for *P. asper* and 32-88 mm TL for *P. tenuis* (Figure 8d).



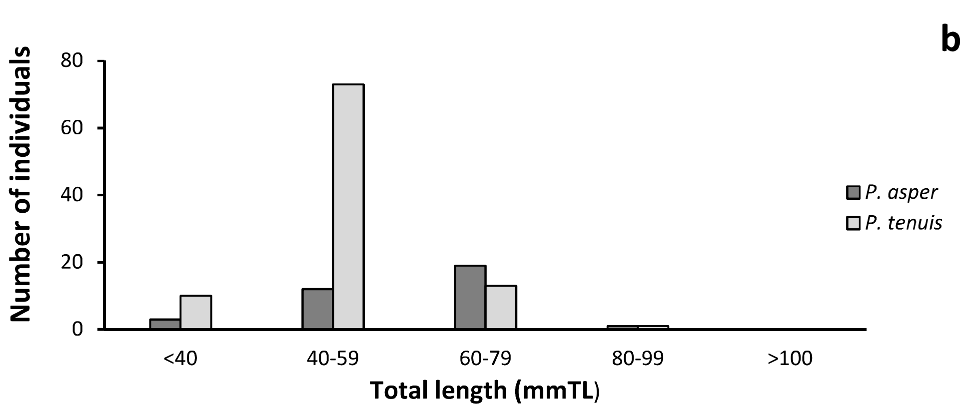
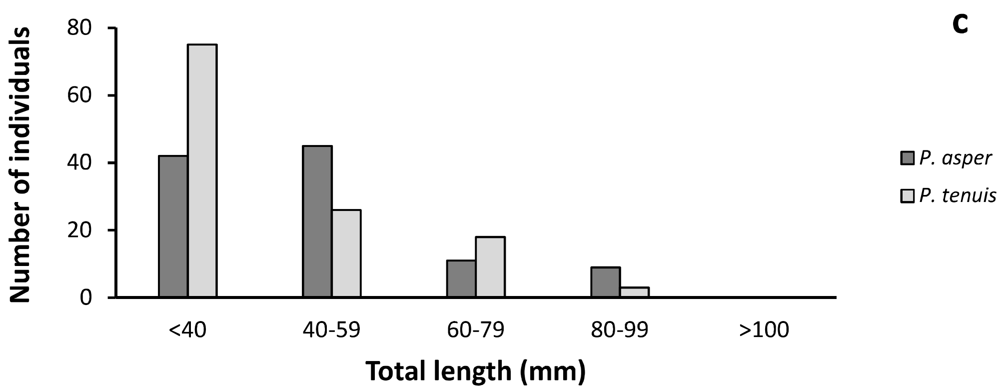




**Figure 8a-b**: Size class distribution of *P. asper* and *P. tenuis* collected from the impacted zone of the Groot River during (a) September 2017 and (b) October 2017.



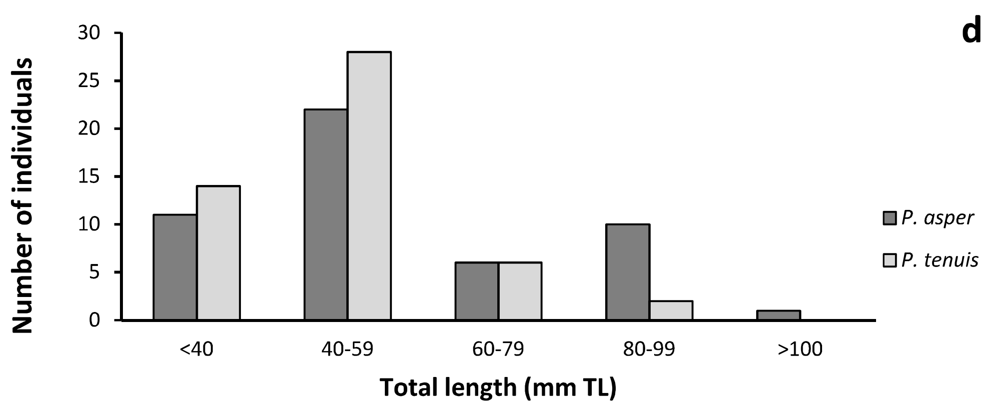




**Figure 8c-d:** Size class distribution of *P. asper* and *P. tenuis* collected from the impacted zone of the Groot River during (c) June 2018 and (d) October 2018.

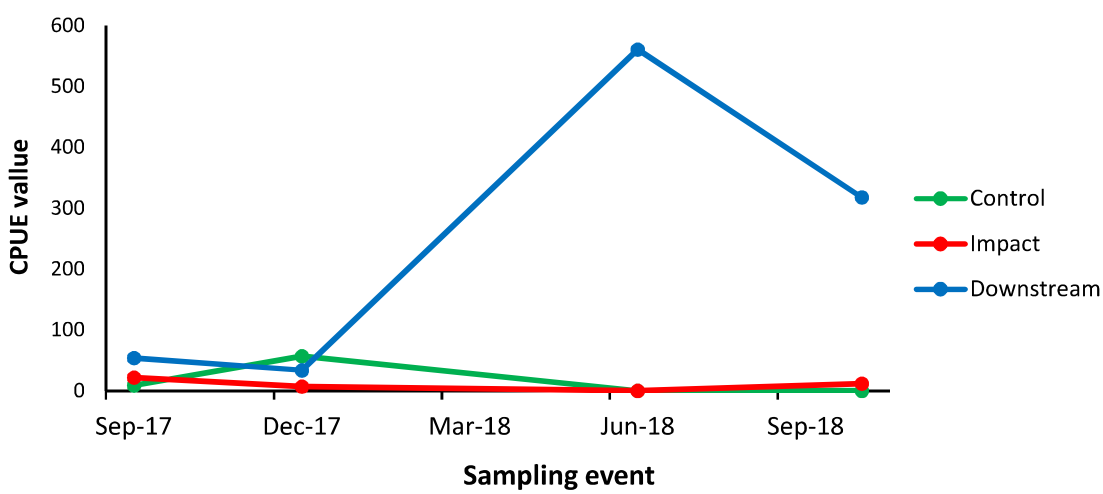



**Figure 9:** Mean catch per unit effort (CPUE) for all three zones of the Groot River for the four sampling intervals

## Discussion and Conclusion

The aim of the present study was to monitor the short-term effects of a diesel spill on the fish community of the Groot River within the Groot Swartberg Nature Reserve and World Heritage site. The Groot River is of high biodiversity value as it is home to three indigenous cyprinid species, one (*P. asper*) of which is listed as Vulnerable (Jordaan and Chakona, 2018). Despite its high biodiversity value and being partially located within a formally protected area, the Groot River is extremely vulnerable to anthropogenic impacts, mainly related to a national road running through the reserve along the river. The risk of point-source pollution related to the use of the road was illustrated in 2017 with the spill of large amounts of diesel into the river following a truck accident. Clean-up and remediation efforts were underway within a very short time (<48h) after the accident which included containment berms, removal of contaminated soils and pumping out of diesel floating on the water surface. The visible impacts of the spill was contained to approximately 900m below the point of entry of the spill into the river. Despite this, significant concerns exist regarding the potential impacts on the aquatic ecosystem of the Groot River.

Fossil fuels such as crude oil and diesel are considered significant global pollutants in both freshwater and marine ecosystems. Sources include ruptured transport pipelines, leakage from storage tanks, storm water drainage and spillage from transport vehicles (Simonato et al. 2011). It is estimated that approximately 5 million tons of crude oil from a variety of sources enters the marine environment each year (Neff, 1990). While an estimation of the pollution load for fossil fuels in freshwater is not known, significant spills and resultant fish kills have been reported. For example, Kubach et al. (2011) reported a diesel spill of 3 624 910 L diesel fuel into the Reedy River, South Carolina from a damaged pipeline which resulted in a 37km fish kill in the river. Diesel is a complex mixture of numerous chemicals including water-soluble polycyclic aromatic hydrocarbons (PAHs) i.e., benzene, toluene, ethylbenzene and xylene (BTEX hydrocarbons), and polyaromatic hydrocarbons such as naphthalene and phenanthrene. Diesel has water soluble fraction (WSF) and water-insoluble fraction (WIF). The WSF is of concern as it is readily bioavailable to aquatic biota and can result in mortality at high concentrations. Sub-lethal effects of the WSF that has been observed in fish include oxidative stress, abnormal liver enzyme activity, imbalance in ion homeostasis, pericardial oedema and spinal deformities in juvenile fish and reduced immune response (Schein et al. 2009). Furthermore, PAHs can combine with the particulate organic or inorganic matter on the stream bed, resulting in increased consumption of oxygen by bacteria during decomposition, thereby reducing available O2 to aquatic biota (Crunkilton & Duchrow 1990). PAHs can also bind to food or sediment particles, therefore short and long lasting biological effects of oil spills are likely to occur (Neff et al.,1976). Fishes are considered particularly vulnerable to polycyclic aromatic hydrocarbons (PAHs) and other persistent contaminants due to their high position in the aquatic food web (Baird and Burton, 2001). Other impacts of diesel contamination can include changes in diatom and invertebrate communities. The latter was investigated during following the spill event through the SASS5 invertebrate sampling methodology and will be reported separately (J. Gouws, unpublished data).

During the short-term observations (<7days) following the spill event, observed mortality of native fish was low (n<25). The clean-up crew was requested to report and collect any dead animals the spill zone, and field rangers walked along the riverbanks in the spill zone every two days. Only a dead duck and dead frog were reported and it is uncertain whether these mortalities can be attributed to the diesel spill. The first dying and moribund fish were observed at roughly four days after the spill in a medium sized pool in the spill zone. The delayed toxic response could be attributed the diesel film preventing oxygen exchange with the atmosphere, thereby causing oxygen depletion and associated mortality, the water soluble fraction of diesel accumulating to acutely toxic concentrations over time, or a combination of these factors. The affected fish were observed in the first approximately 200 m of where the spill occurred and no individuals affected in a similar way were observed at the lower end of the impact zone.

Following the first sampling event conducted two weeks after the spill (September 2017), native redfin species were detected in all three zones sampled. *Pseudobarbus asper* was detected in all three zones sampled while *P. tenuis* was only present at the downstream sampling sites. There were prominent differences in fish abundances between the sites, with the lowest CPUE (9 fish/netting effort) recorded in the control zone and the highest (54 fish/netting effort) in the downstream zone (Figure 9). It is evident that fish abundances increase when moving downstream, but this is likely strongly influenced by the changes in habitat and river size when moving downstream. Redfins were still present in the impacted zone, but fewer fish were detected near the upper end of the spill zone. Fish collected from these two sites were lethargic and showed no avoidance response to being netted or handled when being measured. The netting effort near the end of the spill zone (I3) yielded high numbers of *P. asper* and the behaviour of these fish were markedly different from the fish at sites I1 and I2 in that they showed normal avoidance and startle response to netting and handling. This is likely indicative of less pollutant being present towards the lower end of the spill zone, but this cannot be concluded in the absence of analytical data on diesel pollution concentrations.

*Pseudobarbus tenuis* was only detected at the downstream sampling sites and were absent from the control and impacted sites, with the exception of the December 2017 sampling of site I2 (Figure 7b). This is unlikely to be a result of the diesel contamination. Historical distribution data (CapeNature State of Biodiversity database) indicate that this species has a different distribution range from *P. asper* in the Groot River. Their discontinuous distribution is likely determined by habitat and water quality characteristics in the river, which in turn is determined by the underlying geology of the area. Geologically, the catchments that make up the Groot River is a complex mixture of arenite (a sandstone type of rock formation) in the upper and lower sections of the river and a shale band running through the central section of the Groot River upstream of the reserve border. Downstream sections of the Groot River close to the confluence with the Olifants there are also sections dominated by conglomerates. The downstream zone has markedly different habitat characteristics from the control and impacted zones (see Section 3.1). It must be noted that *P. tenuis* was detected in the Aaps River, a tributary of the Groot River upstream of the Meiringspoort, in a 2016 survey (Jordaan et al. unpublished data) and in the upper Groot River upstream of the Meiringspoort by Swartz (2005). This presents evidence for a naturally discontinuous distribution within the Groot River, most likely driven by this species' habitat preference for clear cold mountain stream habitat (Skelton, 2001).

In terms of population size structure observed in the September 2017 sample event, the redfin population for both the control and impacted zones were dominated by larger size classes (>60mm TL) with a few young fish (<40mm TL) observed in the downstream zone (Figures 5a, 7a and 8a). This is expected as most *Pseudobarbus* redfins commence spawning activity in the spring and the timing of sampling would thus have been prior to the commencement of spawning. Cambray (1994) studied the reproduction biology of the *P. asper* and its sister species, the Eastern Cape redfin (*Pseudobarbus afer*) in the greater Gamtoos River System. It was reported that *P. asper* exhibited a protracted spawning season, ranging from October to April and that spawning is induced by increased river flows. From the December 2017 survey, evidence of successful spawning activity was observed in the control zone, as the majority of fish sampled was in the young of year (<40mm TL) size class ((Figure 5b). Less animals were observed in this size class in the impacted and downstream zones (Figures 7b and 8b), presenting limited evidence for successful recruitment and a possible impact resulting from the diesel spill. However, when considering the June 2018 data from the downstream zone however, it is evident that the young of year size class (Figure 7c) dominate the population of both redfin species. Data from October 2018 shows evidence for ongoing successful spawning activity and high survival of young fish, as the population is dominated by fish of less than 60cm TL. The reduced fish numbers in the impact zone must be interpreted with the fish salvage operations in mind.

The impact of drought is very evident in both the control and the impacted zones and this confounds the impacts of the diesel spill. The control zone started to dry up during December 2017 and was completely dry during the June 2018 survey (Figure 3). Despite reduced flow and associated impacts on habitat, the control zone had the highest CPUE value of all three zones (Figure 9) during December 2017 (Figure 9). This can largely be attributed to successful spawning, illustrated by the dominance of the young of year size class detected during sampling (Figure 5b). It is very likely that some if not all of these fish have perished as a result of this section of river drying up completely during the winter of 2018. While the river was flowing again in October 2018, pool volume was still reduced compared to September 2017 and only two fish were caught, illustrating the severe effects of intermittent droughts on the distribution of threatened native fishes of the region. The few fish observed in the control zone in October 2017 has likely recolonised this section of river from upstream sources (upper Groot River and Aaps River) where populations of both redfin exist (Jordaan and Gouws, 2017). The upstream source populations of redfins are also likely to be the source of the *P. tenuis* individuals that was observed in the impacted zone of the Groot River during the December 2017 survey. When considering the CPUE and size distribution data collected from the downstream zone for all four sample events, there is no evidence to date that suggests significant impacts on the fish community downstream of the spill zone. While a reduction in CPUE was observed between the September 2017 and December 2017 sample events, a CPUE of over 500 fish/netting effort was observed for the June 2018 sample event. The fish that were sampled was also dominated by young of year and juvenile size classes (<60mm TL), presenting evidence for successful recruitment during the past spawning season.

In summary, from the results of this survey, and especially the data from downstream sampling sites, it can be concluded that the Groot River has significant and conservation-worthy populations of two native redfins *P. asper* and *P. tenuis* and that these have not suffered significant deleterious effects from the diesel spill of August 2017. Based on survey work conducted in the Gouritz system between 2015 and 2016, it would appear that *P. asper* has lost the majority of its native range in the Gouritz system to the impacts of alien invasive fish and water over abstraction. This also appears to be the case for the Gamtoos system (Jordaan and Chakona, 2018). The Groot River population is at present one of the biggest *P. asper* populations in the system and has extremely high conservation value. In addition to the threats of pollution and water abstraction, the presence of the highly invasive sharptooth catfish *Clarias gariepinus* was confirmed in the Groot River in 2017 by an independent researcher (R. van der Walt, unpublished data). It appears that *C. gariepinus* have penetrated a significant distance upstream into the poort as this species was detected at Aalwynsdrift, which is a relatively short distance downstream of the Swartberg Reserve border. This species was not detected in downstream sections of the river (Spookdrift bridge) in the direction of the town of De Rust during the 2016 survey of the Swartberg Nature Reserve, but their presence was reported in Stompdrift Dam by several sources (A. Chakona, pers. comm., T. Barry, pers. comm.). An illegal introduction into Stompdrift Dam is the likely source of the invasion. Alarmingly, *C. gariepinus* was also reported to be present in farm dams in the Klaarstroom area towards the northern end of the poort (R. van der Walt, pers. comm). *Clarias gariepinus* is widespread and established in many mainstream river systems in the CFE where there is evidence that their impacts are severe ( Ellender et al (2015) illustrated that *C. gariepinus* has the ability to invade headwater streams but their impact on these fragile ecosystems remains largely unstudied. The *C. gariepinus* invasion into the Groot River warrants urgent attention in the form of monitoring to provide a basis for developing management interventions.

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