Field Report
Aquatic surveys for Grootvadersbosch Conservancy

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1. Introduction

The Grootvadersbosch (GVB) Conservancy area falls into the Langeberg mountain range and is located within the Cape Fold Ecoregion (CFE; Abell *et al.* 2008) and more locally, the transition area between the Southern Coastal Belt and the Southern Folded Mountain ecoregion, Level 1 (Kleynhans *et al.* 2005). The Langeberg mountain range is a prominent feature in the landscape and consists mostly of arenite (Table Mountain sandstones), while the surrounding lower-lying areas have a mostly shale geology. The vegetation types within the Conservancy area is a mosaic of three main types, including Langeberg Sandstone fynbos, which include the Southern Afromontane forests, in the mountainous areas. To the south, the dominant vegetation type is Swellendam Silcrete Fynbos, with Eastern Ruens Shale Renosterveld being present in the lower lying areas. Of these, the renosterveld vegetation type is the most threatened with a threat status of Critically Endangered. The greater mountain catchment area is also considered to be one of the national Strategic Water Source Areas (SWSA; WWF 2013 a&b; Le Maitre *et al.* 2018). Moreover, the GVB Conservancy area is located within parts of the upper catchments of two major river systems, namely the Breede and Duiwenhoks River systems. The Grootvadersbosch River joins with the Tradouw River (becoming the Buffeljags River) and then flows into the Breede River.

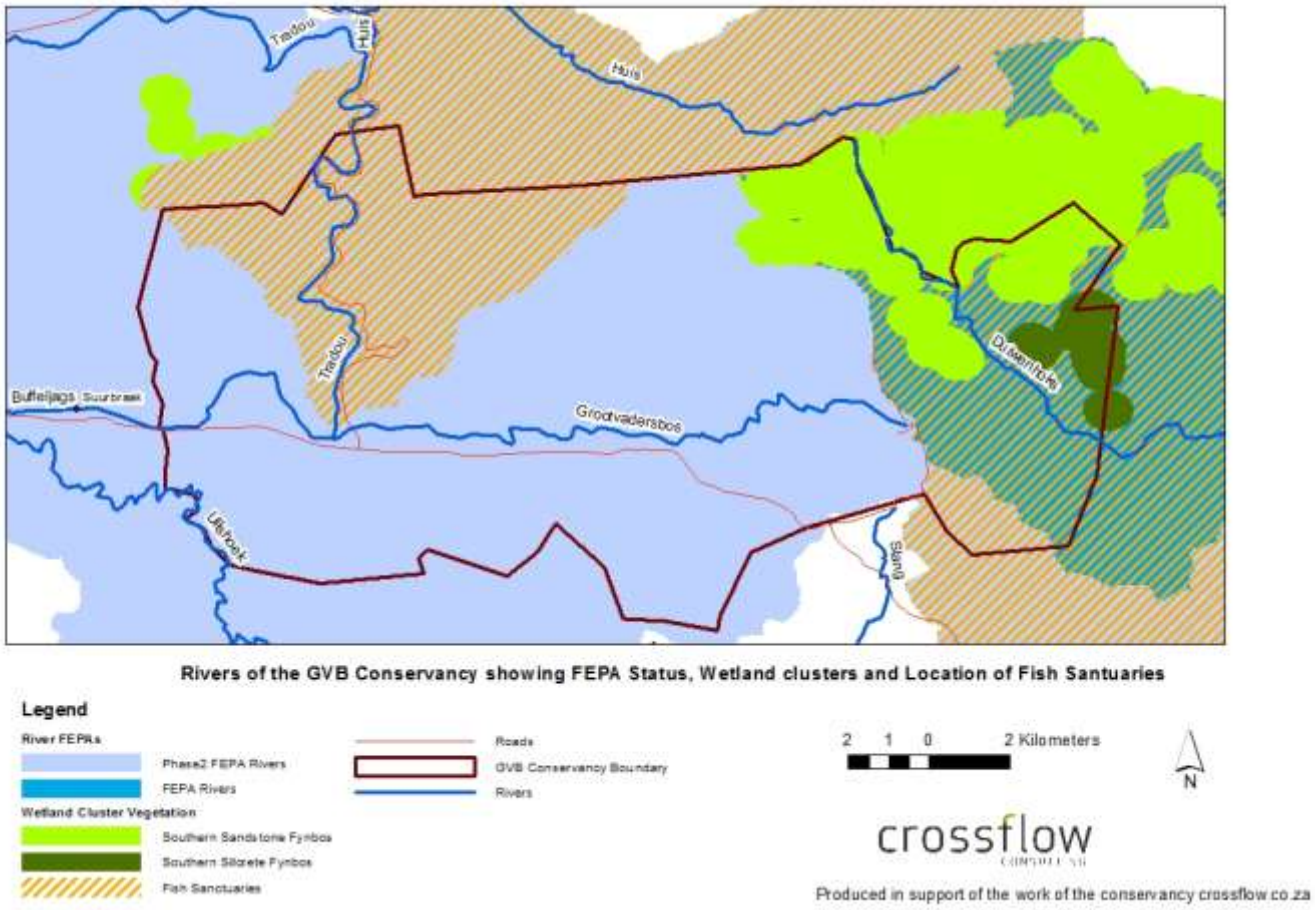

Figure 1: Map of the rivers within the conservancy. The conservancy boundary has now expanded to beyond Suurbraak and this map will be soon be updated to reflect that.

The area receives an average of about 900mm of rainfall a year and while rain is common throughout the year, spring months (October and November) normally have the highest rainfall. The environmental factors in the area, together with the underlying geology and fynbos vegetation types result in slightly peat-stained, fast-flowing mountain streams with cobble beds, dense riparian zones and closed or semi-closed canopies.

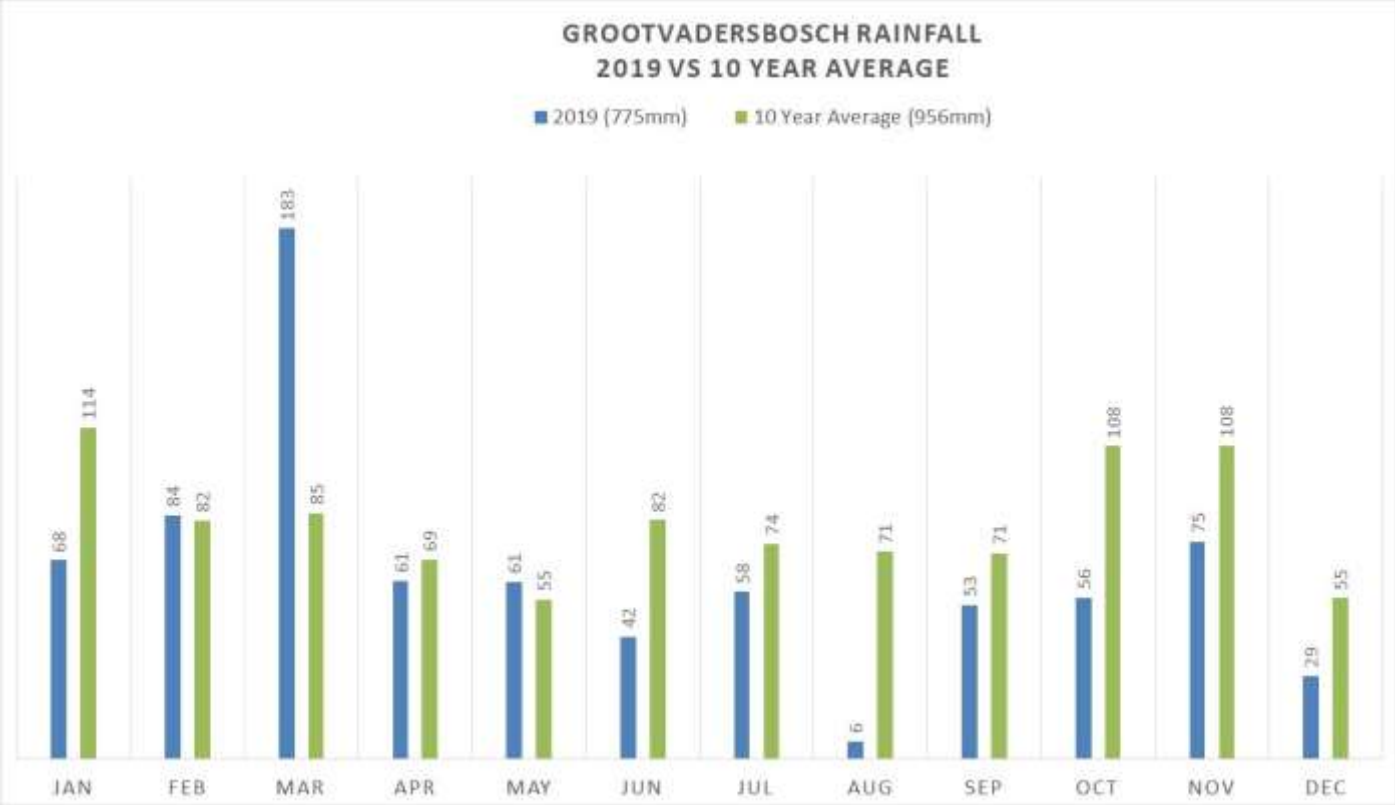

Figure 2: Rainfall figures for the area in 2019 (blue bars) versus the 10-year average (green bars).

A number of alien invasive plants are common in the project area and many rivers, especially those outside of protected areas, have riparian zones dominated by non-native plants. These plants include wattle (*Acacia spp.*), pines (*Pinus spp*.), Hakea (*Hakea spp.*). Starting in 2014, with financial support from landowners and government, the conservancy has cleared about 40km of the riparian areas of the Grootvadersbosch River and supporting tributaries. The alien clearing operations means that natural riparian vegetation in is now returning. The image below show the status of the Grootvadersbosch River in 2014. Similarly, alien and invasive fish species are present in many of the rivers in the region and often dominate foothill and mainstream river reaches. The extent of alien invasion into the rivers of the conservancy is presently unknown and determining the extent of this threat is one of the objectives of the project.

Figure 3: The status of the Grootvadersbosch River in 2014, illustrating the extent of invasive vegetation in the riparian zone of the river prior to the clearing activities.

2. Freshwater fish of the Grootvadersbosch Conservancy Area

2.1 Species diversity and conservation status

The Breede and Duiwenhoks systems are home to a number of currently described indigenous freshwater fish species from four families. Taxonomic research has indicated that many of the currently described indigenous fish species of the greater CFE consist of a number of genetically unique lineages, which confirms the suggestion by Linder *et al.* (2010) that the current taxonomy vastly underestimates the diversity of freshwater fishes of the region. In a recent review by Ellender *et al.* (2017), the current taxonomic richness of the CFR is reported to be 42 unique taxa (described species and know unique lineages). The majority of these unique lineages await taxonomic description as new species and should in the meantime be managed and conserved as unique taxa (Swartz 2005; Skelton and Swartz 2011; Chakona *et al.* 2013). The Breede and Duiwenhoks systems have relatively similar fish faunas. Historically these systems were home to three native species, namely the Breede river redfin *Pseudobarbus burchelli*, the Cape galaxias *Galaxias zebratus* and the Cape kurper *Sandelia capensis*. In addition, the Berg-Breede whitefish *C. capensis* is present in the Breede

River system but absent from the Duiwenhoks and Goukou systems. Recent research has presented evidence that the former three species each represent a species complex. Swartz *et al.* (2009) presented the first evidence that the currently described *Pseudobarbus burchelli,* which occur in the Breede and associated river systems in the Western Cape Province, is a species complex consisting of four genetically distinct lineages. These are a widespread lineage in the Breede, Duiwenhoks and Goukou River systems (*Pseudobarbus* sp. nov. 'Breede'), a lineage in the Heuningnes River system on the Agulhas Plain (*Pseudobarbus* sp. nov. 'Heuningnes') and a lineage restricted to the Tradouw catchment in the eastern section of the Breede River system (*Pseudobarbus* sp. nov. 'Tradouw'). The latter lineage has provisionally been designated as *Pseudobarbus burchelli* sensu stricto as type material for the species was collected form the Tradouw catchment. The fourth lineage has been described as the Giant redfin *Pseudobarbus skeltoni*, (Chakona *et al.* 2013), currently known to be restricted to three localities in the upper Riviersonderend (Kadye *et al.* 2016).

Similarly, Chakona *et al.* (2013) elucidated the presence of several unique lineages within both *G. zebratus* and *S. capensis*. Of these, at least four lineages of galaxias are present in the Breede (*Galaxias* sp. nov. 'breede', *Galaxias* sp. nov. 'nebula', *Galaxias* sp. nov. 'rectognatus' and *Galaxias* sp. nov. 'riviersonderend'). There is ongoing research to resolve the taxonomy of *S. capensis*, but Chakona *et al.* (2013) presented evidence for a lineage within this complex that is restricted to the Breede, Duiwenhoks and Goukou systems. The conservation status of all species and lineages associated with the GVB Conservancy is summarised in Table 1.

A number of alien and invasive fish species are present in the greater Breede and Duiwenhoks systems. These include species both from outside the country as well as species native to the country but alien to the region. Invasive species from outside South Africa include two salmonids (rainbow trout *Oncorhynchus mykiss* and brown trout *Salmo trutta*), four centrarchids (black bass species, namely largemouth bass *Micropterus salmoides*, smallmouth bass *M. dolomieu* and spotted bass *M. punctulatus,* as well as bluegill sunfish *Lepomis macrochirus*) and a single cyprinid the common carp *Cyprinus carpio*. Mozambique tilapia *Oreochromis mossambicus*, sharptooth catfish *Clarias gariepinus* and banded tilapia *Tilapia sparrmanii* are all native to South Africa but alien and invasive in the rivers of the Western (Skelton 2001). Alien and invasive species are widespread throughout the Breede system but the extent of their invasion into the Duiwenhoks system is not as well researched. Where they have invaded, rainbow and brown trout favour cooler mountain streams, with black bass, tilapia and common carp being more common in the warmer lower altitude sections of rivers.

Table 1: Conservation status, main threats and distribution of freshwater fish of the Breede and Duiwenhoks and River systems within the Cape Fold Ecoregion. Data from Skelton (2001), Chakona *et al.* (2013) and Ellender *et al*. (2017).

Species/Taxon	IUCN status/ category	Reference	Main threat	Distribution
Family Anguillidae				
Longfin eel *Anguilla mossambica*	LC	Jacoby and Gollock 2014	0	Kenya to Cape Agulhas, also Madagascar
Family Cyprinidae				
Berg-Breede River whitefish *Cheilobarbus capensis*	EN	Impson et al. 2017	1,2,4,5	Breede River system
Barrydale redfin *Pseudobarbus burchelli* sensu stricto	CR	Jordaan & Chakona 2017a	1,2,3	Tradouw River catchment
Breede River redfin *Pseudobarbus* sp. nov. 'Breede'	VU	Jordaan & Chakona 2017b	1,2	Tributaries of Breede, Duiwenhoks and Goukou systems
Heuningnes redfin *Pseudobarbus* sp. nov. 'Heuningnes'	CR	Chakona & Jordaan 2016	1,2	Heuningnes catchment
Giant redfin *Pseudobarbus skeltoni*	EN	Chakona et al. 2017	1	Three localities in the upper Riviersonderend
Family Galaxiidae				
Cape galaxias *Galaxias zebratus#*	DD	Swartz et al. 2007	1,2,5	Type locality uncertain and required revision#
Galaxias sp. nov.'Breede'	EN	Chakona & Jordaan, 2017*	1,2	Hex and Bothaspruit Rivers, mainstream Breede River
Galaxias sp. nov. 'nebula'	NE	-	-	Widespread across CFR from Olifants to Bietou systems
Galaxias sp. nov. 'rectognatus'	NE	-	-	Riviersonderend sub-catchment of Breede system
Galaxias sp. nov. 'Riviersonderend'	VU	Chakona 2017	1,2	Tributaries of Riviersonderend, also in Breede system
Family Anabantidae				
Cape kurper *Sandelia capensis#*	DD	Chakona 2018	1,2,5	Type locality uncertain and required revision#
Sandelia sp. nov. 'Breede'	NE	-	-	Tributaries of Breede, Duiwenhoks and Goukou systems

Key: CR = Critically Endangered, EN = Endangered, NT = Near Threatened, LC = Least Concern, DD = Data Deficient, NE = Not Evaluated.
Main threats: 0 = no dominant threat identified, 1 = alien fish, 2 = habitat destruction, 3 = pollution, 4 = utilization, 5 = genetic integrity (risk of hybridization or mixing of genetically distinct lineages) in the CFR, South Africa.

These two taxa are undergoing taxonomic revision. The currently described *Galaxias zebratus* occurs from the Olifants-Doring system in the west of the region to the Gamtoos system in the east. *Sandelia capensis* occurs from the Verlorenvlei catchment in the west to the Algoa Bay system in the east. It is naturally absent from the Olifants-Doring system in the west of the region but has some extralimital populations in that system.

Assessments published on the SANBI and IUCN websites: http://speciesstatus.sanbi.org/ and www.iucnredlist.org

2.2 Threats to freshwater fish

Freshwater fishes and their associated ecosystems 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, Dudgeon *et al.* 2006, De Moor and Day 2013). Of these, the presence of non-native fish species is considered the greatest threat to native fishes of the CFE, followed by the loss of habitat (Skelton 1983; Tweddle *et al.* 2009; Weyl *et al.* 2014). Non-native species affect indigenous fishes through predation, habitat alteration, competition for resources, the introduction of diseases and the disruption of ecological processes (Skelton 1987, De Moor and Bruton 1988). The primary impact is predation on smaller species and on juveniles of larger species and this has resulted in the extirpation of most indigenous species from mainstream rivers and tributaries (Weyl *et al.* 2014). Almost all viable populations of indigenous species are now limited to upper reaches of tributaries above waterfalls and other barriers where alien species cannot invade (Skelton, 2001 Chakona *et al.* 2013; Jordaan *et al.* 2012). In addition, population growth and the concomitant increase in water demand for both urban and agricultural use is placing extreme pressure on rivers and other water resources through over abstraction and pollution.

3. Freshwater macro-invertebrates

Mountainous and upland catchment areas are generally considered important not only for the provision of good quality water and as refuges for indigenous fish species, but also because of the substantial contributions they make to biodiversity (Furse 2000; Dallas and Day 2007). This is especially prevalent in the more naturally acidic and low nutrient headwaters of rivers in the Cape Floristic Region, including those within the Grootvadersbosch Conservancy area. These conditions that have resulted in high aquatic species richness and also high degrees of endemism in the CFR as a whole (Gouws and Gordon 2017; de Moor and Day 2013). There is also a high level of genetic diversity within several invertebrate taxa (i.e. taxonomic disparity; de Moor and Day 2013) as is also the case for indigenous fish species. This so-called taxonomic disparity has resulted in the concept of “catchment signatures” with regard to the invertebrate assemblages present in the different river catchments of the CFR (see King and Schael 2001; Dallas and Day 2007). With the levels of sensitivity that is linked to many of the endemic invertebrate taxa within these catchment signature assemblages, it is not surprising that this faunal group has been used extensively as indicators of river health (see Dickens and Graham 2002). Despite this, not enough is currently known about all the invertebrate taxa that might occur in the CFR river systems, including the rivers of the GVB Conservancy (de Moor and Day 2013). Only a subset of groups have been studied and there is limited understanding what the effect of anthropogenic activities are on these taxa and their patterns of community and endemism.

Biomonitoring of foothill and headwater streams, such as those found within the GVB Conservancy area can be used to establish the reference/benchmark conditions for a river system that might be impacted on locally or in the lowland areas. Here benthic macro-invertebrates can be used to monitor both water quality and habitat diversity over the long term, using the South African Scoring System version 5 (SASS 5) method (Dickens and Graham 2002).

4. Site selection and sampling methodology

Sample sites were selected with the focus on rivers that were highlighted as fish sanctuaries on the NFEPA maps and rivers within the conservancy area where historical data was absent. Locality data of all sampling sites completed to date are listed in Table 2. Two field trips were conducted to date, one in December 2018 and the second one in February 2020. Fish were sampled using fyke nets (60 x 60 cm square fyke with 2mm mesh side and side panels of 5m); and a seine net (2 x 10 m net with 2mm mesh). Fyke nets were deployed overnight and soak time was standardized to 14 hours. Habitat characteristics were noted at each site and water quality parameters (pH, temperature, conductivity and dissolved oxygen) were recorded. At each site all species caught (alien and indigenous), size class distribution and relative abundance was recorded. Voucher specimens and DNA samples were collected where appropriate and lodged in the national fish collection at the South African Institute for Aquatic Biodiversity (SAIAB).

Where there was flowing water, the ecological integrity of the river was analysed though the SASS 5 methodology following standardized protocols (Dickens and Graham 2002). The SASS 5 method is considered a rapid bio-assessment method and is used to assess the water quality, habitat availability and health of a river system (Dickens and Graham 2002). The method uses the presence/absence of macroinvertebrate families to evaluate water quality, where a sensitivity/tolerance score out of 15 is linked to each taxon. The higher the score, the more sensitive the specific taxon is to pollution. The method, to a degree, also takes invertebrate abundance into account as well as habitat (or biotope) availability, as different taxa prefer different parts of a river system. This method has been used extensively (e.g. River Health Programme) and is considered cost effective and time efficient. Here, different macro-invertebrate taxa are given a score out of 15, with higher scores being related to more sensitive (in terms of water quality impairments) taxa, and lower scores relating to taxa that are more tolerant to pollution. The final scores take into account the sum of the scores per taxon (SASS Score) observed and the number of different taxa, from where an Average Score Per Taxon (ASPT) is calculated. Both the SASS Score and the ASPT is then used to determine the health of a river site or system, through the ecological banding system that was developed by Dallas (2007). These two scores are plotted against each other (see the figure for the Southern Coastal Belt and Southern Folded

Mountains – upper and lower in Dallas 2007) and each point falls into an ecological category, ranging from natural (A) to critically modified (E/F).

Table 2: Summary of sampling sites and GPS localities for the study

Site Code	River	DDS	DDE
BR-28	Huis - upstream	-33,9037	20.7250
BR29	Huis - downstream	-33.9102	20.7132
BMP-01	Tradouw	-33.9537	20.7078
H8DUIW-00001	Duiwenhoks – western tributary	-33.9834	20.8453
H8DUIW-00003	Duiwenhoks – eastern tributary	-34.0062	20.8856
H8DUIW-00002	Duiwenhoks – downstream	-34.0062	20.8856
H8KLIP-00001	Noukrans	-33.9805	20.8677
H7TRAD-00003	Tradouw – upstream of waterfall upper	-33.9764	20.6983
H7TRAD-00004	Tradouw – upstream of waterfall middle	-33.9815	20.7076
H7TRAD-00005	Tradouw – lower	-33.9974	20.7033
H7KRUI-00002	Kruis - lower	-33.9991	20.7311
H7KRUI-00003	Kruis - upper	-33.9889	20.7482
H7GROO-00001	Grootvadersbosch - middle	-34.0035	20.7948
H7GROO-00002	Grootvadersbosch - lower	-34.0077	20.7020
H7GROO-00003	Grootvadersbosch - upper	-33.9973	20.7983
H7GATB-00001	Gatboskloof	-33.9842	20.7195
H7CALE-00001	Caledon	-33.9957	20.6627
H7TRIB-00001	Rietvalleikloof (tributary of Buffeljags)	-33.9989	20.6047

4.1 2018 Survey results

4.1.1 Upper Huis River (H7HUIS-00001)

<table>
<tr><td>GPS</td><td colspan="2">S: 33.903722^{O}</td><td colspan="2">E: 20.725^{O}</td><td>Sampling date:</td><td>23/11/2018</td></tr>
<tr><td>Site description:</td><td colspan="6">The river was sampled in a pool immediately downstream of a water abstraction weir in the river. Only slow-deep habitat was present and very little flow entered the pool as the majority of the water was abstracted into the offtake canal. The pool had extensive marginal vegetation in the form of palmiet beds and substrate consisted mainly of bedrock and some cobble. The water was peat stained but clear. Submerged root wads from the palmiet beds provided fish habitat in the absence of undercut banks and woody debris. A sparse wattle infestation is present in the riparian zone but has been identified for follow-up clearing. This site represent one of the sites for long term monitoring in the Tradouw system.</td></tr>
<tr><td rowspan="2">Water Quality:</td><td>pH</td><td>DO (%)</td><td>DO (mg/L)</td><td>Temp (°C)</td><td colspan="2">Conductivity (mS)</td></tr>
<tr><td>5.58</td><td>-</td><td>6.6</td><td>21.4</td><td colspan="2">0.0596</td></tr>
<tr><td>Sampling methodology</td><td colspan="6">Fyke net – 1 effort</td></tr>
<tr><td>Fish species present</td><td colspan="6">The only species present was the Barrydale redfin P. burchelli sensu stricto. All size classes were present (n=49, size range: 45-120mm TL) and many fish had well developed tubercles, indicative of recent spawning activity. Large adult fish dominated the population with relatively few juveniles observed.</td></tr>
<tr><td>SASS5 Biotopes sampled</td><td colspan="6">Stones in Current, Gravel Sand and Mud, Aquatic Vegetation</td></tr>
<tr><td rowspan="2">Scores</td><td colspan="2">No of taxa collected</td><td colspan="2">SASS score</td><td colspan="2">ASPT score</td></tr>
<tr><td colspan="2">18</td><td colspan="2">102</td><td colspan="2">5.67</td></tr>
<tr><td>SASS notes</td><td colspan="6">Palmiet, sedges, grasses and Isolepis sp. present. Cape river frog tadpoles moderately abundant.</td></tr>
<tr><td>Site photo</td><td colspan="6">  </td></tr>
</table>

4.1.2 Lower Huis River (H7HUIS-00002)

<table>
<tr><td>GPS</td><td colspan="2">S: 33. 910222^{O}</td><td colspan="2">E: 20.71327778^{O}</td><td>Sampling date:</td><td>23/11/2018</td></tr>
<tr><td>Site description:</td><td colspan="6">The downstream sampling site in the Huis River was located next to the Barrydale Winery and consisted of a single pool upstream of an instream gauging weir. There was very little flow at the time of sampling and the majority of habitat consisted of slow-deep and slow-shallow habitat. Substrate was a combination of bedrock, cobbles and sand. Submerged vegetation was abundant in shallower areas and marginal vegetation was present in the form of reeds and sedges on the right bank. The water was both peat stained and moderately turbid, likely a result of upstream water abstraction disrupting normal flow patterns. This site represent one of the sites for long term monitoring in the Tradouw system.</td></tr>
<tr><td rowspan="2">Water Quality:</td><td>pH</td><td>DO (%)</td><td>DO (mg/L)</td><td>Temp (ºC)</td><td colspan="2">Conductivity (mS)</td></tr>
<tr><td>6.09</td><td>-</td><td>6.6</td><td>19.7</td><td colspan="2">0.184</td></tr>
<tr><td>Sampling methodology</td><td colspan="6">Fyke net – 1 effort</td></tr>
<tr><td>Fish species present</td><td colspan="6">Two fish species were detected, namely the Barrydale redfin P. burchelli and banded tilapia T. sparrmanii. The redfins were abundant and all size classes were present (n=150, size range: 38-100mm TL). No fish with tubercles were observed and the subsample of redfins that were measured (n=40) were severely infested with trematode parasites.Banded tilapia were abundant (n=227) and while size measurements were not recorded, all size classes were present.</td></tr>
<tr><td>SASS5 Biotopes sampled</td><td colspan="6">Stones in Current, Gravel Sand and Mud, Aquatic Vegetation</td></tr>
<tr><td rowspan="2">Scores</td><td colspan="2">No of taxa collected</td><td colspan="2">SASS score</td><td colspan="2">ASPT score</td></tr>
<tr><td colspan="2">17</td><td colspan="2">70</td><td colspan="2">4.12</td></tr>
<tr><td>SASS notes</td><td colspan="6"></td></tr>
<tr><td>Site photo</td><td colspan="6">  </td></tr>
</table>

4.1.3 Tradouw River (H7TRAD-00002)

<table>
<tr><td>GPS</td><td colspan="2">S: 33.9573^{O}</td><td colspan="2">E: 20.7078^{O}</td><td>Sampling date:</td><td>23/11/2018</td></tr>
<tr><td>Site description:</td><td colspan="6">The Tradouw River was sampled within the Tradouw Gorge and consisted of a series of deep pools with mainly bedrock and cobble substrate. Marginal and instream vegetation was absent and an infestation of wattle was observed in the riparian zone. All velocity-depth classes were present but habitat was dominated by the slow-deep class. The water was peat-stained but clear and more flow was observed than at the upstream Huis River sites.</td></tr>
<tr><td rowspan="2">Water Quality:</td><td>pH</td><td>DO (%)</td><td>DO (mg/L)</td><td>Temp (°C)</td><td colspan="2">Conductivity (mS)</td></tr>
<tr><td>6.65</td><td>-</td><td>7.1</td><td>23.0</td><td colspan="2">0.237</td></tr>
<tr><td>Sampling methodology</td><td colspan="6">Fyke net – 2 efforts</td></tr>
<tr><td>Fish species present</td><td colspan="6">Three species were detected, namely redfins P. burchelli, banded tilapia T. sparrmanii and the longfin eel A. mossambica. The redfins were less abundant than at the upstream Huis River sites but all size classes were present (n= 39, size range: 48-118mm TL). Some fish with tubercles were observed and no sign of trematode parasites were detected. Banded tilapia were also not abundant (n=24) and while size measurements were not recorded, all size classes were present. Three eels, two sub-adult and one adult, were observed. Results from previous surveys indicated that largemouth bass M. salmoides and bluegill L. macrochirus is present in areas upstream from the sampling site (Jordaan et al. unpublished data).</td></tr>
<tr><td>SASS5 Biotopes sampled</td><td colspan="6">Stones in Current, Gravel Sand and Mud, Aquatic Vegetation</td></tr>
<tr><td rowspan="2">Scores</td><td colspan="2">No of taxa oberved</td><td colspan="2">SASS score</td><td colspan="2">ASPT score</td></tr>
<tr><td colspan="2">21</td><td colspan="2">124</td><td colspan="2">5.9</td></tr>
<tr><td>SASS notes</td><td colspan="6">Aquatic vegetation consisted mostly of sedges.</td></tr>
<tr><td>Site photo</td><td colspan="6">  </td></tr>
</table>

4.1.4 Duiwenhoks River – western tributary (H8DUIW-00001)

<table>
<tr><td>GPS</td><td colspan="2">S: 33.9834^{O}</td><td colspan="2">E: 20.8453^{O}</td><td>Sampling date:</td><td>7/12/2018</td></tr>
<tr><td>Site description:</td><td colspan="6">The western tributary of the Duiwenhoks River was sampled in the headwaters with the sampling site located a short distance downstream of the reserve boundary of the Boosmansbos Wilderness Area. The river was flowing moderately well at the time of sampling and the water was clear but peat-stained. All velocity-depth classes were present and the substrate consisted of boulders, rocks and cobbles as well as some bedrock in deeper areas. While the riparian vegetation was largely natural at the sampling site, a significant wattle invasion, coupled with agricultural impacts, was observed downstream and in the immediate catchment area.</td></tr>
<tr><td rowspan="2">Water Quality:</td><td>pH</td><td>DO (%)</td><td>DO (mg/L)</td><td>Temp (°C)</td><td colspan="2">Conductivity (mS)</td></tr>
<tr><td>5.63</td><td>-</td><td>9.2</td><td>18.6</td><td colspan="2">0.107</td></tr>
<tr><td>Sampling methodology</td><td colspan="6">Fyke net – 1 effort</td></tr>
<tr><td>Fish species present</td><td colspan="6">Only Cape kurper S. capensis was detected and all size classes were present except young of year (n=40, size range: 55-114 mm TL). The presence of Cape galaxias G. zebratus was reported close to the sampling site reported by earlier studies (Chakona et al. 2013) and this was confirmed by the detection of G. zebratus during the SASS sampling.</td></tr>
<tr><td>SASS5 Biotopes sampled</td><td colspan="6">Stones in Current, Gravel Sand and Mud, Aquatic Vegetation</td></tr>
<tr><td rowspan="2">Scores</td><td colspan="2">No of taxa collected</td><td colspan="2">SASS score</td><td colspan="2">ASPT score</td></tr>
<tr><td colspan="2">20</td><td colspan="2">140</td><td colspan="2">7.0</td></tr>
<tr><td>SASS notes</td><td colspan="6">Evidence of fire in area and riparian zone (1 month prior), lots of leaf litter instream. Collected 1 Sandelia capensis (sub adult) and 2 G. zebratus in Stone sample. Ptylodactilidae larvae collected in Veg & Gravel and Sand samples.</td></tr>
<tr><td>Site photo</td><td colspan="6">  </td></tr>
</table>

4.1.5 Duiwenhoks River – eastern tributary (H8DUIW-00003)

<table>
<tr><td>GPS</td><td colspan="2">S: 34.0062^{O}</td><td colspan="2">E: 20.8856^{O}</td><td>Sampling date:</td><td>7/12/2018</td></tr>
<tr><td>Site description:</td><td colspan="6">The site was sampled at a pool at a road crossing. Apart from the pool habitat, no open water was visible and the entire river was covered by dense palmiet stands. The water was clear at the time of sampling with some visible flow downstream of the bridge. The substrate consisted of very deep mud that made netting difficult. Slow-deep and slow-shallow was the only velocity depth classes present at the time of sampling. The dense palmiet stands made root wads abundant, but undercut banks and woody debris was absent. There was also abundant marginal vegetation in the form of sedges at the edges of the pool. A medium density wattle infestation was observed in the riparian zone.</td></tr>
<tr><td rowspan="2">Water Quality:</td><td>pH</td><td>DO (%)</td><td>DO (mg/L)</td><td>Temp (°C)</td><td colspan="2">Conductivity (mS)</td></tr>
<tr><td>-</td><td>-</td><td>-</td><td>-</td><td colspan="2">-</td></tr>
<tr><td>Sampling methodology</td><td colspan="6">Seine net – 1 effort</td></tr>
<tr><td>Fish species present</td><td colspan="6">Cape galaxias G. zebratus was the only taxon detected (n=2, adult and subadult)</td></tr>
<tr><td>SASS5 Biotopes sampled</td><td colspan="6">No SASS was conducted as there was no flowing water at the site.</td></tr>
<tr><td rowspan="2">Scores</td><td colspan="2">No of taxa collected</td><td colspan="2">SASS score</td><td colspan="2">ASPT score</td></tr>
<tr><td colspan="2">-</td><td colspan="2">-</td><td colspan="2">-</td></tr>
<tr><td>SASS notes</td><td colspan="6"></td></tr>
<tr><td>Site photo</td><td colspan="6">  </td></tr>
</table>

4.1.6 Duiwenhoks River – downstream site (H8DUIW-00002)

GPS	**S:** 34.0062^{O}	**E:** 20.8856^{O}	**Sampling date:**	7/12/2018
Site description:	The second sampling site on the Duiwenhoks River was located downstream of a road crossing. The river had moderate flow at the time of sampling and all velocity-depth classes were present. The substrate consisted of rocks and cobbles in the riffle areas with some bedrock sheets present in deeper pool areas. Riparian vegetation consisted mainly of palmiet and there was evidence of wattle clearing in the riparian zone. There were extensive amounts of submerged aquatic vegetation present in the shallow sections of the pool and filamentous algae was abundant. There were moderate amount of undercut banks and root wads and some woody debris from alien clearing activities. The water was clear but deeply peat stained and there were periphyton on the rock in slow flowing pool habitat.			

Water Quality:	**pH**	**DO (%)**	**DO (mg/L)**	**Temp (°C)**	**Conductivity (mS)**
	5.7	-	6.2	24	0.085

Sampling methodology	Fyke net – 2 efforts
Fish species present	Two taxa were detected during sampling, redfins *Pseudobarbus* sp. nov. 'breede' (n=51, size range: 54-105mm TL) and Cape kurper *S. capensis* (n=72, size range: 36-91mm TL). Cape galaxias *Galaxias zebratus* were not detected but caught while conducting SASS and were found in the Stones in Current (SIC) biotope. No alien fish were detected.
SASS5 Biotopes sampled	Stones in Current, Gravel Sand and Mud, Aquatic Vegetation

Scores	**No of taxa collected**	**SASS score**	**ASPT score**
	17	92	5.41

SASS notes	Collected *G. zebratus* (juvenile & adult) in stone and vegetation samples. Collected one Ptylodactilidae in Stones and six Cape river frog tadpoles in Vegetation samples.
Site photo	

4.1.7 Noukrans River (H8KLIP-00001)

<table>
<tr><td>GPS</td><td colspan="2">S: 33.9805^{O}</td><td colspan="2">E: 20.8677^{O}</td><td>Sampling date:</td><td>7/12/2018</td></tr>
<tr><td>Site description:</td><td colspan="6">The Noukrans River was sampled downstream of the reserve boundary of the Boosmansbos Wilderness area. The river was flowing well at the time of sampling and all velocity-depth classes were present. A large instream diversion weir was present at the sampling site which was overflowing at the time of sampling. The substrate consisted mainly of rocks and boulders with some bedrock in pool areas. Marginal and overhanging vegetation was abundant on both banks, along with undercut banks and root wads. The water was clear but peat-stained.</td></tr>
<tr><td rowspan="2">Water Quality:</td><td>pH</td><td>DO (%)</td><td>DO (mg/L)</td><td>Temp (°C)</td><td colspan="2">Conductivity (mS)</td></tr>
<tr><td>5.6</td><td>-</td><td>9.6</td><td>18.6</td><td colspan="2">0.076</td></tr>
<tr><td>Sampling methodology</td><td colspan="6">Fyke net – 2 efforts (upstream and downstream of weir)</td></tr>
<tr><td>Fish species present</td><td colspan="6">Only Cape kurper S. capensis was detected and all size classes were present except young of year (n=40, size ran0ge: 54-162 mm TL). No redfins were detected despite their presence being reported by earlier studies (Chakona et al. 2013) and their detection during the present survey in the mainstream Duiwenhoks.</td></tr>
<tr><td>SASS5 Biotopes sampled</td><td colspan="6">Stones in Current, Gravel Sand and Mud, Aquatic Vegetation</td></tr>
<tr><td rowspan="2">Scores</td><td colspan="2">No of taxa collected</td><td colspan="2">SASS score</td><td colspan="2">ASPT score</td></tr>
<tr><td colspan="2">18</td><td colspan="2">142</td><td colspan="2">7.89</td></tr>
<tr><td>SASS notes</td><td colspan="6">SASS site downstream of weir. Six taxa scoring 10 or more collected, including from the stone fly family Notonemouridae (score of 14), more than 2 species of the minnow mayfly family Baetidae (score of 12), the SWC endemic mayfly family Teloganodidae (score of 12), the SWC endemic cased caddisfly families Barbarothonidae and Sericostomatidae (both scoring 13). Habitat diversity was moderate to high.</td></tr>
<tr><td>Site photo</td><td colspan="6">  </td></tr>
</table>

4.2 2020 Survey results

4.2.1 Tradouw River upstream of waterfall upper (H7TRAD-00003)

<table>
<tr><td>GPS</td><td colspan="2">S: 33.976476 O</td><td>E: 20.698331</td><td colspan="2">Sampling date:</td><td>24/02/2020</td></tr>
<tr><td>Site description:</td><td colspan="6">One of three new Tradouw River sites was sampled about 2km upstream of the waterfall in the Tradouw gorge. The river was flowing well at the time of sampling and all velocity-depth classes were present. The substrate consisted mainly of boulders and bedrock with some cobbles in pool areas. Marginal and overhanging vegetation was limited on both banks, along with undercut banks and root wads. Woody debris was sparse. The water was clear but peat-stained.</td></tr>
<tr><td rowspan="2">Water Quality:</td><td>pH</td><td>DO (%)</td><td>DO (mg/L)</td><td>Temp (°C)</td><td colspan="2">Conductivity (mS)</td></tr>
<tr><td>5.37</td><td>12.1</td><td></td><td>22.3</td><td colspan="2">0.0798</td></tr>
<tr><td>Sampling methodology</td><td colspan="6">Fyke net – 1 effort, seine net – 1 effort</td></tr>
<tr><td>Fish species present</td><td colspan="6">Two species were detected, banded tilapia T. sparrmanii and the longfin eel A. mossambica. Two eels, two sub-adult and one adult, were observed. No redfins were detected.</td></tr>
<tr><td>SASS5 Biotopes sampled</td><td colspan="6">Stones in current, some stone out of current, bedrock. Marginal vegetation in current, limited marginal vegetation out of current and aquatic vegetation. Sand and some gravel. Varying flow depths and velocities.</td></tr>
<tr><td rowspan="2">Scores</td><td colspan="2">No of taxa collected</td><td colspan="2">SASS score</td><td colspan="2">ASPT score</td></tr>
<tr><td colspan="2">10</td><td colspan="2">53</td><td colspan="2">5.3</td></tr>
<tr><td>SASS notes</td><td colspan="6">SASS site located in between the two new sites on the Tradouw River upstream of the waterfall. Flow was unusually high. Rain over weekend preceding sampling. Mostly medium to low scoring taxa collected. Highest scoring taxa include more than two species of the minnow mayfly family Baetidae (12), caseless caddisfly family Ecnomidae (8) and beetle family Elmidae (8).</td></tr>
<tr><td>Site photo</td><td colspan="6">  </td></tr>
</table>

4.2.2 Tradouw River upstream of waterfall middle (H7TRAD-00004)

<table>
<tr><td>GPS</td><td colspan="2">S: 33.981573</td><td>E: 20.707597</td><td>Sampling date:</td><td>25/02/2020</td></tr>
<tr><td>Site description:</td><td colspan="5">One of three new Tradouw River sites (middle) was sampled a few hundred meters upstream of the waterfall in the Tradouw gorge. The site is in the vicinity of the “Drupkelder” viewpoint on the road. The river was flowing well at the time of sampling and all velocity-depth classes were present. Slow-deep was the most abundant habitat type with most pools longer than 50m. The substrate consisted mainly of boulders and bedrock with cobbles and gravel in pool areas. Marginal and overhanging vegetation was moderately abundant on both banks, along with undercut banks and root wads. Woody debris was sparse. The water was clear but peat-stained.</td></tr>
<tr><td rowspan="2">Water Quality:</td><td>pH</td><td>DO (%)</td><td>DO (mg/L)</td><td>Temp (°C)</td><td>Conductivity (mS)</td></tr>
<tr><td>-</td><td>-</td><td>-</td><td>-</td><td>-</td></tr>
<tr><td>Sampling methodology</td><td colspan="5">Fyke net – 1 effort, seine net – 1 effort. Effective seine netting was hampered by the rocky substrate and was not pursued as a sampling method in this area.</td></tr>
<tr><td>Fish species present</td><td colspan="5">Only longfin eel A. mossambica were detected. Five eels, one sub-adult and four adults, were observed in the fyke net. No other species were detected but it is possible that if any other fish were caught, the eels would have predated on them.</td></tr>
<tr><td>SASS5 Biotopes sampled</td><td colspan="5">No SASS or water quality assessments were conducted as the site was relatively close to the previous site and habitat was very similar.</td></tr>
<tr><td rowspan="2">Scores</td><td colspan="2">No of taxa collected</td><td>SASS score</td><td colspan="2">ASPT score</td></tr>
<tr><td colspan="2">-</td><td>-</td><td colspan="2">-</td></tr>
<tr><td>SASS notes</td><td colspan="5">-</td></tr>
<tr><td>Site photo</td><td colspan="5"> 
</td></tr>
</table>

4.2.3 Tradouw River (H7TRAD-00005)

<table>
<tr><td>GPS</td><td colspan="2">S: 33.99739^{O}</td><td colspan="2">E: 20.70332^{O}</td><td>Sampling date:</td><td>25/02/2020</td></tr>
<tr><td>Site description:</td><td colspan="6">The furthest downstream site in the Tradouw River was sampled on private land close to the confluence with the Grootvadersbosch River. The river was flowing well at the time of sampling and all velocity-depth classes were present. Substrate consisted mainly of boulders, rocks and cobbles. Marginal and overhanging vegetation was moderately abundant on both banks, along with undercut banks and root wads. The right bank showed signs of bank erosion and wattle trees were abundant. Woody debris was common. The water was clear but peat-stained.</td></tr>
<tr><td rowspan="2">Water Quality:</td><td>pH</td><td>DO (%)</td><td>DO (mg/L)</td><td>Temp (°C)</td><td colspan="2">Conductivity (mS)</td></tr>
<tr><td>4.44</td><td>10.1</td><td>20.3</td><td>0.0798</td><td colspan="2">5.37</td></tr>
<tr><td>Sampling methodology</td><td colspan="6">Fyke net – 1 effort</td></tr>
<tr><td>Fish species present</td><td colspan="6">No fish were caught as the net was damaged extensively while deployed, presumably by otters. Moderate amounts of partially eaten fish and crab remains were observed in the net, indicating fish presence.</td></tr>
<tr><td>SASS5 Biotopes sampled</td><td colspan="6">Stones in current, some stone out of current, bedrock. Marginal vegetation in current, limited marginal vegetation out of current and aquatic vegetation. Sand and some gravel. Varying flow depths and velocities.</td></tr>
<tr><td rowspan="2">Scores</td><td colspan="2">No of taxa collected</td><td colspan="2">SASS score</td><td colspan="2">ASPT score</td></tr>
<tr><td colspan="2">19</td><td colspan="2">120</td><td colspan="2">6.32</td></tr>
<tr><td>SASS notes</td><td colspan="6"></td></tr>
<tr><td>Site photo</td><td colspan="6">  </td></tr>
</table>

4.2.4 Kruis River – downstream site (H7KRUI-00002)

<table>
<tr><td>GPS</td><td colspan="2">S: -33.99913^{O}</td><td colspan="2">E: 20.73113^{O}</td><td>Sampling date:</td><td>25/02/2020</td></tr>
<tr><td>Site description:</td><td colspan="6">The downstream site on the Kruis River was sampled on private land upstream of any human disturbance. The river was flowing well at the time of sampling due to significant rain a few days prior to sampling. All velocity-depth classes were present. The substrate consisted mainly of rocks, cobbles and boulders with some bedrock in pool areas. Marginal and overhanging vegetation was abundant on both banks, along with undercut banks and root wads. The river channel was shaded in some sections because of abundant overhanging vegetation. The water was clear but very peat-stained. Periphyton cover on the rocks was moderate to scare.</td></tr>
<tr><td rowspan="2">Water Quality:</td><td>pH</td><td>DO (%)</td><td>DO (mg/L)</td><td>Temp (°C)</td><td colspan="2">Conductivity (mS)</td></tr>
<tr><td>3.61</td><td>-</td><td>11.6</td><td>18.4</td><td colspan="2">0.0684</td></tr>
<tr><td>Sampling methodology</td><td colspan="6">Fyke net – 1 effort</td></tr>
<tr><td>Fish species present</td><td colspan="6">Indigenous Cape kurper S. capensis was detected from the fyke net in high abundance and with all size classes present. In addition, Cape galaxias G. zebratus was detected in moderate abundance from faster flowing riffle habitat during SASS sampling (n=5). No alien fish were detected during sampling and all fish were in good health.</td></tr>
<tr><td>SASS5 Biotopes sampled</td><td colspan="6">Stones in current, some stone out of current and some boulders. Marginal vegetation in current, some marginal vegetation out of current and some aquatic vegetation. Some sand gravel. Varying flow depths and velocities.</td></tr>
<tr><td rowspan="2">Scores</td><td colspan="2">No of taxa collected</td><td colspan="2">SASS score</td><td colspan="2">ASPT score</td></tr>
<tr><td colspan="2">16</td><td colspan="2">103</td><td colspan="2">6.44</td></tr>
<tr><td>SASS notes</td><td colspan="6">Flow was unusually high. Rain over weekend preceding sampling. Fairly high diversity of taxa collected. Highest scoring taxa include individuals from the Hydracarina (8), the stonefly family Notonemouridae (14), the mayfly family Teloganodidae (SWC endemic; 12), the caseless caddisfly family Philopotamidae (10), the cased caddisfly family Pisulidae (10) and the beetle families Elmidae (8) and Scirtidae (12).</td></tr>
<tr><td>Site photo</td><td colspan="6">  </td></tr>
</table>

4.2.5 Grootvadersbosch River – downstream site (H7GROO-00001)

<table>
<tr><td>GPS</td><td colspan="2">S: -34.004</td><td>E: 20.79479</td><td>Sampling date:</td><td>26/02/2020</td></tr>
<tr><td>Site description:</td><td colspan="5">This site is located on the Grootvadersbosch farm about 800m upstream of farming activities. The river was flowing well at the time of sampling due to overnight rain with an active channel of 2-5m and all velocity-depth classes were present. The substrate consisted mainly of boulders with some bedrock in pool areas. Marginal and overhanging vegetation along with undercut banks and root wads were present in moderate abundance. Woody debris was sparse. The water was clear but very peat-stained.</td></tr>
<tr><td rowspan="2">Water Quality:</td><td>pH</td><td>DO (%)</td><td>DO (mg/L)</td><td>Temp (ºC)</td><td>Conductivity (mS)</td></tr>
<tr><td>4.3</td><td>-</td><td>11.3</td><td>18.0</td><td>0.0805</td></tr>
<tr><td>Sampling methodology</td><td colspan="5">Fyke net - 1 effort, scoop net – several efforts in riffle habitat</td></tr>
<tr><td>Fish species present</td><td colspan="5">Four fish species were detected of which three were indigenous and one was alien. The indigenous fish were the Cape kurper S. capensis (n= 14, size range 32-88mm TL), redfins Pseudobarbus sp. nov. ‘breede’ (n=12, size range 55-93mm TL) and Cape galaxias G. zebratus (all size classes, collected mainly from riffles using scoop net). A single banded tilapia T. sparrmanii (76mm TL) was collected from the fyke net.</td></tr>
<tr><td>SASS5 Biotopes sampled</td><td colspan="5">Stones in current, some stone out of current and some boulders. Marginal vegetation in current, some marginal vegetation out of current and some aquatic vegetation. Some sand gravel. Varying flow depths and velocities.</td></tr>
<tr><td rowspan="2">Scores</td><td colspan="2">No of taxa collected</td><td>SASS score</td><td colspan="2">ASPT score</td></tr>
<tr><td colspan="2">21</td><td>163</td><td colspan="2">7.76</td></tr>
<tr><td>SASS notes</td><td colspan="5">SASS site located in riffles between two pools, just upstream of fish sampling site. Flow high because of rain over weekend preceding sampling. Fairly high diversity of taxa collected. Highest scoring taxa include individuals from the Hydracarina (8), the stonefly family Notonemouridae (14), more than two species of the minnow mayfly family Baetidae (12), the mayfly families Heptageniidae (13) and Leptophlebiidae (9), the Dobson/fish fly family Corydalidae (8), the beetle families Elmidae (8) and Scirtidae (12) and the true fly family Athericidae (10).</td></tr>
<tr><td>Site photo</td><td colspan="5">  </td></tr>
</table>

4.2.6 Grootvadersbosch River (H7GROO-00002)

<table>
<tr><td>GPS</td><td colspan="2">S: 34.0076806^{O}</td><td colspan="2">E: 20.70205^{O}</td><td>Sampling date:</td><td>27/02/2020</td></tr>
<tr><td>Site description:</td><td colspan="6">This site was located upstream of the bridge when entering Tradouw Pass on the farm Hoëkraal. The river was flowing well at the time of sampling due to rain a few days preceding sampling. All velocity-depth classes were present and substrate consisted mainly of boulders with some bedrock in pool areas. Marginal and overhanging vegetation was sparse on both banks, along with undercut banks and root wads. The water was clear but peat-stained. There was evidence that the river banks had been bulldozed recently.</td></tr>
<tr><td rowspan="2">Water Quality:</td><td>pH</td><td>DO (%)</td><td>DO (mg/L)</td><td>Temp (ºC)</td><td colspan="2">Conductivity (mS)</td></tr>
<tr><td>6.06</td><td>-</td><td>8.4</td><td>23.1</td><td colspan="2">0.129</td></tr>
<tr><td>Sampling methodology</td><td colspan="6">Fyke net – 1 effort</td></tr>
<tr><td>Fish species present</td><td colspan="6">No fish detected at time of sampling. Landowners report presence of sharptooth catfish. This species was detected in 2010 upstream of Buffeljags Dam near Suurbraak (unpublished RHP survey data).</td></tr>
<tr><td>SASS5 Biotopes sampled</td><td colspan="6">Stones in current and some stone out of current. Aquatic vegetation and some marginal vegetation in and out of current. Some sand and mud with limited gravel. Varying flow depths and velocities.</td></tr>
<tr><td rowspan="2">Scores</td><td colspan="2">No of taxa collected</td><td colspan="2">SASS score</td><td colspan="2">ASPT score</td></tr>
<tr><td colspan="2">20</td><td colspan="2">111</td><td colspan="2">5.55</td></tr>
<tr><td>SASS notes</td><td colspan="6">The SASS site was located downstream of the fish sampling site about 200m upstream of confluence with Tradouw River. Cape river frog tadpoles were collected in SASS net. Diversity of medium to low scoring taxa collected. Highest scoring taxa include individuals from more than two species of the minnow mayfly family Baetidae (12), the mayfly family Teloganodidae (SWC endemic; 12), the caseless caddisfly family Ecnomidae and the beetle family Elmidae (8).</td></tr>
<tr><td>Site photo</td><td colspan="6">  </td></tr>
</table>

4.2.7 Gatboskloof River (H7GATB-00001)

<table>
<tr><td>GPS</td><td colspan="2">S: 33.98426^{O}</td><td colspan="2">E: 20.719547^{O}</td><td>Sampling date:</td><td>26/02/2020</td></tr>
<tr><td>Site description:</td><td colspan="6">The Gatboskloof River was sampled in the Tradouw Pass at the “Andries Uys Bridge”. The river was flowing well at the time of sampling due to rain a few days preceding sampling and all velocity-depth classes were present. The dominant habitat type was slow-deep as the river consisted mainly of a series of large interconnected pools with dense palmiet stands all along the edges. The substrate consisted mainly of bedrock and a few cobbles and pebbles. Marginal and overhanging vegetation was abundant on both banks, along with palmiet root wads. Undercut banks and woody debris was sparse. The water was clear but peat-stained.</td></tr>
<tr><td rowspan="2">Water Quality:</td><td>pH</td><td>DO (%)</td><td>DO (mg/L)</td><td>Temp (°C)</td><td colspan="2">Conductivity (mS)</td></tr>
<tr><td>6.06</td><td>-</td><td>8.4</td><td>23.1</td><td colspan="2">0.129</td></tr>
<tr><td>Sampling methodology</td><td colspan="6">Fyke net - 1 effort</td></tr>
<tr><td>Fish species present</td><td colspan="6">No fish were detected at time of sampling.</td></tr>
<tr><td>SASS5 Biotopes sampled</td><td colspan="6">Dominated by bedrock. Aquatic vegetation and marginal vegetation in current with limited marginal vegetation out of current.</td></tr>
<tr><td rowspan="2">Scores</td><td colspan="2">No of taxa collected</td><td colspan="2">SASS score</td><td colspan="2">ASPT score</td></tr>
<tr><td colspan="2">10</td><td colspan="2">82</td><td colspan="2">8.2</td></tr>
<tr><td>SASS notes</td><td colspan="6">The SASS site was located in a bedrock cascade just upstream of the second large pool from the bridge. Palmiet (Prionium serratum) dominated the marginal vegetation, along with Isolepis sp. present instream on bedrock. Diversity of medium to low scoring taxa collected. Highest scoring taxa include individuals from the South Western Cape (SWC) endemic mayfly family Teloganodidae (12), the cased caddisfly families Barbarochtonidae (SWC endemic; 13) and Pisulidae (11) and the beetle families Elmidae (8) and Scirtidae (12).</td></tr>
<tr><td>Site photo</td><td colspan="6">  </td></tr>
</table>

4.2.8 Kruis River (H7KRUI-00002)

GPS	**S:** 33.9889^{O}		**E:** 20.74821^{O}	**Sampling date:**	27/02/2020
Site description:	The upper Kruis River was sampled on Glen Etive farm upstream of series of pools, cascades and waterfalls and upstream of all human disturbance. The river was flowing well at the time of sampling and all velocity-depth classes were present. The substrate consisted mainly of large bedrock sheets with some rocks and boulders in pool areas. Marginal and overhanging vegetation comprised indigenous species and was relatively abundant on both banks along with some root wads. Undercut banks and woody debris was less common. The water was clear but peat-stained.				
Water Quality:	**pH**	**DO (%)**	**DO (mg/L)**	**Temp (ºC)**	**Conductivity (mS)**
	-	-	-	-	-
Sampling methodology	Fyke net - 1 effort				
Fish species present	No fish were detected at time of sampling.				
SASS5 Biotopes sampled	SASS assessment and water quality measurements outstanding & scheduled for next survey				
Scores	**No of taxa collected**		**SASS score**	**ASPT score**	
	-		-	-	
SASS notes					
Site photo					

4.2.9 Caledon River (H7CALE-00001)

<table>
<tr><td>GPS</td><td colspan="2">S: 33.995681^{O}</td><td colspan="2">E: 20.662718^{O}</td><td>Sampling date:</td><td>18/03/2020</td></tr>
<tr><td>Site description:</td><td colspan="6">The Caledon River is a tributary of the Buffeljags River and was sampled upstream of a large instream weir close to the town of Suurbraak. The river was flowing well at the time of sampling and all velocity-depth classes were present. The right bank consisted of a sandstone cliff face and deep habitat while the left bank had a gentle slope and consisted of boulders and rocks. The substrate was a combination of bedrock, boulders, rocks and cobbles. Marginal and overhanging vegetation was absent on the left bank and sparse to moderate on the right bank. The riparian vegetation consisted mainly on invasive black wattle but some native species were still present. Undercut banks and root wads were sparse and woody debris sparse to moderate. The water was clear but very peat-stained.</td></tr>
<tr><td rowspan="2">Water Quality:</td><td>pH</td><td>DO (%)</td><td>DO (mg/L)</td><td>Temp (°C)</td><td colspan="2">Conductivity (mS)</td></tr>
<tr><td>-</td><td>-</td><td>-</td><td>-</td><td colspan="2">-</td></tr>
<tr><td>Sampling methodology</td><td colspan="6">Fyke net - 1 effort</td></tr>
<tr><td>Fish species present</td><td colspan="6">Only Cape kurper S. capensis were detected (n=9, size range 94-142 mm TL) along with six adult platannas Xenopus laevis.</td></tr>
<tr><td>SASS5 Biotopes sampled</td><td colspan="6">SASS assessment and water quality measurements outstanding & scheduled for next survey.</td></tr>
<tr><td rowspan="2">Scores</td><td colspan="2">No of taxa collected</td><td colspan="2">SASS score</td><td colspan="2">ASPT score</td></tr>
<tr><td colspan="2">-</td><td colspan="2">-</td><td colspan="2">-</td></tr>
<tr><td>SASS notes</td><td colspan="6"></td></tr>
<tr><td>Site photo</td><td colspan="6">  </td></tr>
</table>

4.2.10 Rietvalleikloof River (Buffeljags tributary) (H7TRIB-00001)

<table>
<tr><td>GPS</td><td colspan="2">S: -33.998986°</td><td>E: 20.604686°</td><td colspan="2">Sampling date:</td><td>18/03/2020</td></tr>
<tr><td>Site description:</td><td colspan="6">This tributary of the Buffeljags River was sampled on the farm Rietvallei upstream of human disturbance. The river was flowing very well at the time of sampling and all velocity-depth classes were present. The sampling site comprised a large pool (20x20m) with a bedrock waterfall at the inlet and riffles at the outlet. The substrate was mainly bedrock and boulders with some rocks and cobbles downstream. Undercut banks and root wads were sparse to moderate and comprised mainly palmiet stands at the edge of the pool. Woody debris was sparse but observed to be more abundant in downstream areas. The riparian zone was mostly native but evidence of large-scale wattle clearing was observed.</td></tr>
<tr><td rowspan="2">Water Quality:</td><td>pH</td><td>DO (%)</td><td>DO (mg/L)</td><td colspan="2">Temp (°C)</td><td>Conductivity (mS)</td></tr>
<tr><td>-</td><td>-</td><td>-</td><td colspan="2">-</td><td>-</td></tr>
<tr><td>Sampling methodology</td><td colspan="6">Fyke net - 1 effort</td></tr>
<tr><td>Fish species present</td><td colspan="6">The only fish sampled were Cape galaxias G. zebratus that was present in low numbers (n=3) and varying size classes.</td></tr>
<tr><td>SASS5 Biotopes sampled</td><td colspan="6">SASS assessment and water quality measurements outstanding & scheduled for next survey.</td></tr>
<tr><td rowspan="2">Scores</td><td colspan="2">No of taxa collected</td><td colspan="2">SASS score</td><td colspan="2">ASPT score</td></tr>
<tr><td colspan="2">-</td><td colspan="2">-</td><td colspan="2">-</td></tr>
<tr><td>SASS notes</td><td colspan="6"></td></tr>
<tr><td>Site photo</td><td colspan="6">  </td></tr>
</table>

4.2.11 Grootvadersbos River (H7GROO-00003)

<table>
<tr><td>GPS</td><td colspan="2">S: -33.997266°</td><td>E: 20.798311°</td><td colspan="2">Sampling date:</td><td>19/03/2020</td></tr>
<tr><td>Site description:</td><td colspan="6">The upstream site on the Grootvadersbos River was sampled on private land downstream of the Grootvadersbos Nature Reserve. Land use at the sampling site consisted of conservation and very low-density cattle grazing. The river was flowing well at the time of sampling with a channel width of 3-5m. All velocity-depth classes was present with the absence of fast-deep. The riparian zone comprised mainly natural vegetation. Overhanging vegetation were abundant on both banks, along with woody debris. Undercut banks and root wads were present in moderate amounts. The water was clear but very peat-stained.</td></tr>
<tr><td rowspan="2">Water Quality:</td><td>pH</td><td>DO (%)</td><td>DO (mg/L)</td><td>Temp (°C)</td><td colspan="2">Conductivity (mS)</td></tr>
<tr><td>-</td><td>-</td><td>-</td><td>-</td><td colspan="2">-</td></tr>
<tr><td>Sampling methodology</td><td colspan="6">Fyke net - 1 effort, scoop net – several efforts in riffle habitat</td></tr>
<tr><td>Fish species present</td><td colspan="6">Three fish species were detected, all of whom are indigenous. These were the Cape kurper S. capensis (n=56, size range 45-118 mm TL), Pseudobarbus sp. nov. ‘breede’ (n=1, 70 mm TL) and Cape galaxias G. zebratus (abundant, all size classes, collected mainly from riffles using scoop net). No alien fish were detected.</td></tr>
<tr><td>SASS5 Biotopes sampled</td><td colspan="6">SASS assessment and water quality measurements outstanding & scheduled for next survey.</td></tr>
<tr><td rowspan="2">Scores</td><td colspan="2">No of taxa collected</td><td colspan="2">SASS score</td><td colspan="2">ASPT score</td></tr>
<tr><td colspan="2">-</td><td colspan="2">-</td><td colspan="2">-</td></tr>
<tr><td>SASS notes</td><td colspan="6"></td></tr>
<tr><td>Site photo</td><td colspan="6">  </td></tr>
</table>

5. Results and Discussion

5.1 Fish

Nineteen sites were surveyed for the present study of which four were located in the upper Duiwenhoks River system and the remaining 15 in the Breede River system. All the expected native fish taxa were detected (Figure 5a-c), with the most commonly detected species being the Cape kurper *S. capensis* that was present at 37% of sites (n=7 sites). The Cape galaxias *G. zebratus* was also relatively common and present at 32% of sites (n=6 sites). The two redfin taxa that is present in the rivers of the conservancy, namely the Barrydale redfin *P. burchelli* sensu stricto and the Breede River redfin *Pseudobarbus* sp. nov. 'breede' were both present at 16% of sites (n=3 sites). This data is presented in Figure 6. Of the 19 sites surveyed, no fish were detected at four of them (Tradouw – lower, Kruis – upper, Gatboskloof and Grootvadersbos – lower). Of these, the non-detection of fish in the lower Tradouw and lower Grootvadersbos rivers is unexpected as historical records exist for native fish in these rivers, both rivers have viable fish populations upstream and anecdotal records exist of invasive species such as sharptooth catfish. The non-detection of fish from the lower Tradouw River should be seen as a false negative as otters destroyed the net during deployment and thus no data could be collected. Fish remains, which resembled the dorsal fin of a sharptooth catfish, was observed in the net. Sharptooth catfish are present in the Buffeljags River as juvenile individuals were sampled near Suurbraak during a River Health Programme survey in 2009 (Jordaan and Impson, unpublished data). As the lower Tradouw site is located relatively close to the confluence with the Grootvadersbos River, the presence of catfish can be inferred in this river as there are no physical barriers that would prevent fish movement. For these reasons, urgent further surveys are required to determine the fish community of these river sections.

The other two sites where fish were not detected were the upper Kruis and the Gatboskloof. Of these, it is likely that the site on the upper Kruis River is located upstream of the natural fish distribution range for this river. Cape kurper were collected at the downstream sampling site in this river and their upstream distribution limit for this river is unknown and warrants further investigation. Cape kurper can be found in a wide range of habitats but favour quiet or slow-flowing water with plant or root cover (Skelton 2001). Chakona and Swartz (2012) investigated the causal factors that influence the spatial distribution of fish in the greater Breede River system and reported that elevation and slope were of primary importance for Cape kurper. These authors reported that in their study, this species was not found in reaches that had channel slopes greater than 15 m/km and elevation higher than 425 m, and were primarily associated with pool habitat.

Chakona and Swartz (2012) proposed that the habitat preference of Cape kurper is related to its morphological characteristics. Cape kurper have laterally compressed bodies, large pectoral fins and square shaped caudal fins, which translate to reduced swimming performance due to high drag penalties. Cape kurper may therefore struggle to maintain position under greater turbulence, due to increased energetic demands, thus explaining why this species was absent from reaches at higher elevations and steeper gradients. While data on slope and altitude were not collected during this study, it is likely that the habitat of the upper Kruis River is unsuitable to Cape kurper and that their presence is limited to suitable habitat in downstream sections of the river

In contrast to the Cape kurper, the slender and small-bodied Cape galaxias should be able to utilize habitat in high elevation streams and steep gradients, which are characterised by strong currents and turbulent flow. In addition, many headwater streams are small and shallow and only provide habitat for very small-bodied fish such as Cape galaxias. Chakona and Swartz (2012) illustrated this as they reported that galaxias often penetrated into reaches at higher elevation than either Cape kurper of Breede river redfins. While evidence for extended distribution ranges for Cape galaxias were not observed in the present study (i.e. they mostly co-occurred with other native fish), this is likely a result of limited sampling of headwater environments. The preference of Cape galaxias for faster flowing habitats such as riffles and runs were observed for both the lower Kruis and the Grootvadersbos Rivers. Here Cape galaxias were absent from (or present in very low abundances) in the fyke nets set in pool habitat but commonly detected during SASS sampling that focused on faster flowing habitat. This was also observed during many other surveys in rivers of the CFE (Jordaan et al. 2016; Jordaan and Gouws 2017, 2019). Given the evidence for the role of habitat parameters as drivers for freshwater fish distributions, the reason for the observed absence of fish in the Gatboskloof River is not straightforward. While suitable fish habitat for all native fish species exist in the river, a small waterfall and a series of cascades exist close to the confluence of the Gatboskloof and Tradouw Rivers. This is however unlikely to present a fish barrier. More sampling should be conducted to ensure that the absence of fish is not merely an artefact of the low sampling effort. When considering Cape galaxias distribution throughout the conservancy, it is evident that this species is well represented in the rivers of the area. They are present in both upper tributaries of the Duiwenhoks River, as well as the Grootvadersbos and Kruis rivers. It is expected that further sampling of suitable habitat in the Caledon River should confirm their presence, as only pool habitat was sampled and not riffle habitat as well. Their absence in the Noukrans River was unexpected and further sampling here may elucidate their presence.

Figure 5a: Breede River redfin *Pseudobarbus* sp. nov. 'Breede' (left) and *Pseudobarbus burchelli* sensu stricto (right)

Figure 5b: Cape galaxias *Galaxias zebratus* (left) and Cape kurper *Sandelia capensis* (right)

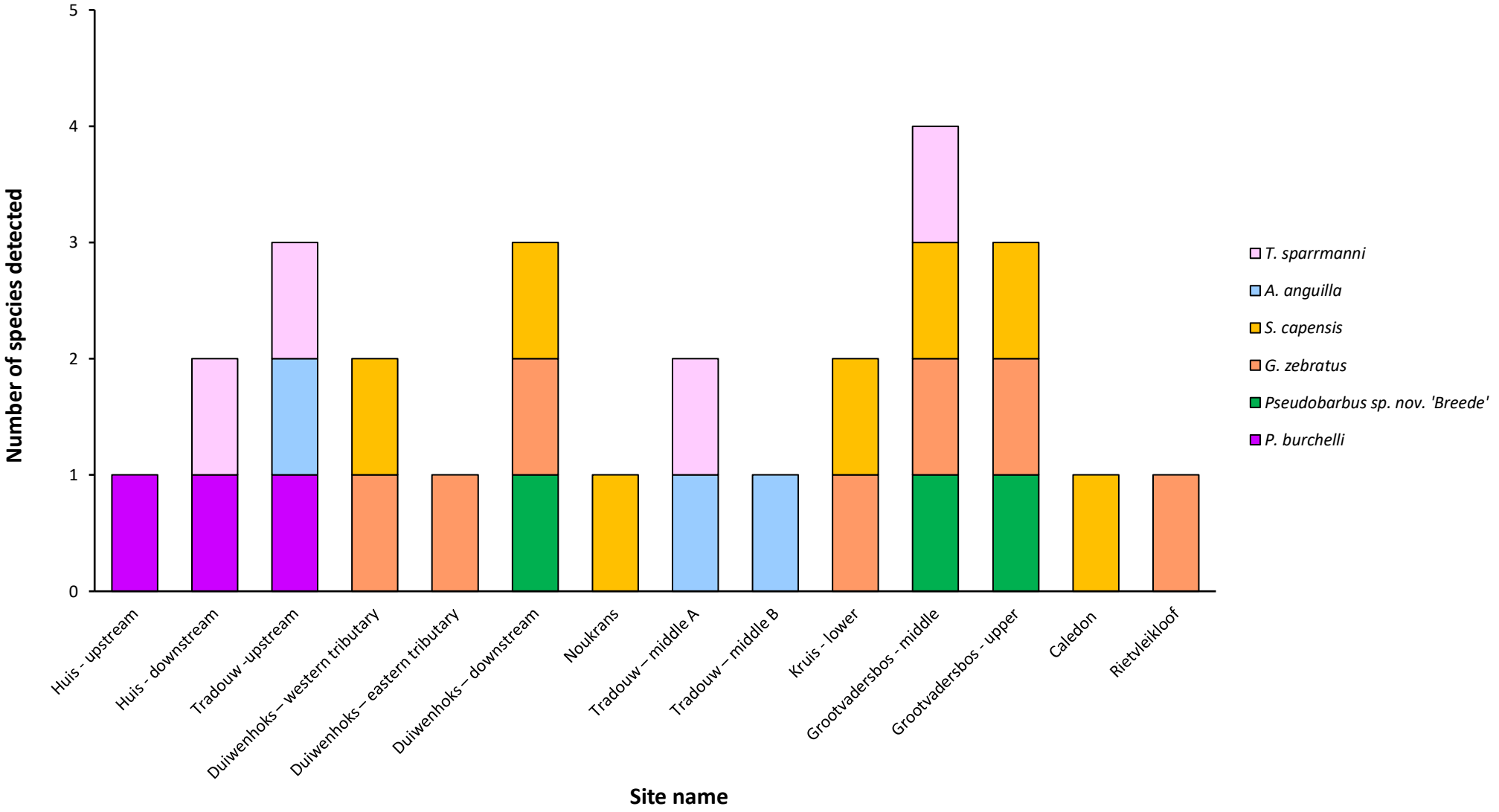

Figure 6: Data summary of species composition detected at each of the sampling sites. The four sites where no fish were detected, namely the Tradouw – lower, Kruis – upper, Gatboskloof and Grootvadersbos – lower, were excluded.

In terms of the Critically Endangered *Pseudobarbus burchelli*, thee sites with viable populations were sampled during the survey. Of these, two are in the Huis River in Barrydale and is thus outside the conservancy area while the population in the Tradouw Pass is within the boundary of the conservancy. Monitored annually since 2012, there is evidence that population in the Tradouw River is highly variable in terms of size and population structure, while the population in in the Huis River upstream of Barrydale appears more stable (Jordaan *et al*. unpublished data). The main threat to the redfins in the Huis River is water over-abstraction for both agricultural and residential use, which results in the river becoming stagnant with poor water quality during the dry months. This adversely affects the redfin through loss of habitat and increased incidence of diseases such as black spot. Improved management of water demand is key to conserving this redfin population in the long term, as is clearing of invasive pants and managing agricultural and urban pollution. The Tradouw River redfin population is threatened by the upstream presence of non-native fish including black bass, bluegill sunfish and banded tilapia. Additional sampling sites were explored downstream of the annual sampling site but no redfins were detected. This likely does not represent a true absence but is rather a reflection of sampling methodology. Seine netting was rendered ineffective by the very rocky substrate and moderately strong flow during sampling and the very high relative abundance of eels posed a risk to redfin caught in fyke nets. Alternative sampling methods such as snorkeling, Go-Pro sampling and electrofishing was not attempted due to the low visibility associated with deeply peat-stained rivers. Obtaining accurate population estimates for the redfins in the Tradouw River remains problematic. Options to explore going forward could include deploying fyke nets during the day when eels are less active and experimenting with including a mesh cover over the open end of the fyke to discourage large eels from entering the net but still allowing the smaller-bodied redfins to enter the net.

The negative effects of eel predation on the redfins in the Tradouw River must be seen in context, as eels are indigenous to the greater Breede River system and are natural predators of native fish such as redfins. Eels are also good indicators of river health and connectivity, as they need to migrate in order to complete their life cycle. The eels observed in the Tradouw pass would have migrated from the ocean via the Breede River estuary as juveniles (so-called glass eels) before moving to freshwater and becoming elvers. These elvers migrate further into freshwater environments where they stay for several years before undergoing several physio-morphological changes to become the sea-going form (so-called silver eels) and returning to the ocean as adults to breed (Skelton 2001). The vulnerability of freshwater eels on their perilous migration between freshwater and marine environments is clear when considering the impacts on freshwater ecosystems which include water over-abstraction, impoundments (such as the Buffeljags Dam), instream structures (such as weirs) and pollution. This is exacerbated by occasional targeting by both recreational and

subsistence anglers. The presence of relatively large numbers of eels, all in a healthy condition, in the Tradouw River is a positive sign in terms of river health and connectivity.

Both the upper Grootvadersbos River and the headwaters of the Duiwenhoks River are in good health in terms of the indigenous fish community. All three expected species were detected in good numbers and all size classes were present. The confirmation of a healthy indigenous fish population in the upper Grootvadersbos River is a positive result from this study, as the last record for redfin from this river was reported in 1986 (CapeNature internal SOB database). The sections of river where the redfins were sampled is also upstream of previous records and close to the boundary with Grootvadersbos Nature Reserve. In the case of Cape kurper, historical records do exist for close to where the river was sampled but date back to 1978. These populations were not included in recent Red List Assessments but the updated information can now be included in follow-up assessments. The native fish community in in the Duiwenhoks River is also healthy and indigenous species were abundant with all size classes present, confirming the distribution records from Chakona *et al*. (2013). The project also yielded three new distribution records. No records were found for Cape kurper and Cape galaxias in the lower Kruis River in either the CapeNature or SAIAB databases. The Cape kurper records in the Noukrans is also new, as is the Cape galaxias records from the upper Grootvadersbos River. Possibly, local knowledge of these populations have been present for quite some time, but this project enable formal sampling which include the collection of voucher specimens and DNA samples for inclusion in the National Fish Museum at SAIAB. The collection of DNA and voucher material is especially important given that both the Cape kurper and Cape galaxias is currently undergoing taxonomic revision and fine scale collection of sampled supports this research.

No new invasive fish records were detected during the surveys with the exception of a single banded tilapia *T. sparrmanii* at middle site sampled on the Grootvadersbos River. This species is well established in the Huis River in Barrydale and throughout the Tradouw River in the Tradouw gorge. This is likely the invasion source, given the absence of invasion barriers. While not detected in the lower Tradouw and lower Grootvadersbos River, it is unlikely that this species is absent from these regions and it is expected that further sampling will confirm their presence. The native range of banded tilapia is from the Orange River and Kwazulu-Natal south coast northwards to the upper reaches of tributaries in the southern Congo, Lake Malawi and the Zambezi system (Skelton 2001). It has been extensively translocated in the CFE and is present in all primary river systems of the Western Cape (Jordaan *et al*. 2012). The non-detection of sharptooth catfish thus far was unexpected given that they have been detected in the Buffeljags River in 2009 (Jordaan and Impson, unpublished data). Sharptooth catfish are indigenous to the Afrotropical regions, where it occurs from the Nile to as far south as the Orange and Umtamvuna River systems in South Africa (Cambray 2003). This species

is an emerging global invader (Cambray 2003; Vitule *et al.* 2006; Radhakrishnan *et al.* 2011) that since the 1960s, has invaded several rivers in the CFR following direct introductions and escape from aquaculture facilities (Cambray 2003). Invasions are of concern because this species is able to penetrate previously uninvaded threatened fish refugia in headwater streams (Ellender *et al*. 2015) and because it has been demonstrated that sharptooth catfish introductions within newly invaded habitats resulted in negative impacts on aquatic invertebrate communities (Kadye *et al.* 2012). Their impacts on native fish communities in their invasive range is poorly studied but expected to be significant. The invasive success and associated impacts of sharptooth catfish on aquatic ecosystems can be attributed to a range of factors, including their ability to survive in and adapt to various environmental conditions, their ability to survive desiccation, their omnivorous feeding habits, high fecundity and fast growth rate (Cambray 2003). Jordaan *et al*. (2016) also illustrated that this species has the ability to survive high concentrations of the piscicide rotenone, thus limiting the application of piscicides to manage invasive populations. Determining the invasion status of this species into the Grootvadersbos River and setting up long term monitoring to identify and understand the impacts of the invasion should be prioritized during this survey and into the future.

5.2 SASS 5

The sampling sites were located within the transition zone of the Southern Coastal Belt Level 1 Ecoregion to the south and the Sothern Folded Mountains Level 1 Ecoregion to the north. Furthermore, most sites were located within the mountain stream and upper foothill reaches of the rivers, with four sites reaches of the rivers (i.e. lower sites on the Duiwenhoks, Grootvadersbosch, Huis and Tradouw rivers). The river water was generally naturally discoloured (tea stained colour). Instream habitat diversity, namely the composition and distribution of instream and marginal stones, vegetation, sediments and flow velocity, was generally found to be moderate to high. Consequently, in most cases, all three SASS 5 biotopes (stones, vegetation and gravel-sand-mud) were present to some degree at the survey sites. For many of the sites, the flow was moderate to high due to rainfall events in the catchments. Several precipitation events had also taken place on a regular basis since November 2019. High flow conditions are not considered ideal for SASS sampling, however, the results obtained are still meaningful. Consequently, the results were analysed taking into account both ecoregion divisions as well as whether the sites were located in the upper or lower reaches of the rivers (see Figures 7 and 8).

As expected, the sites with the highest SASS results were those located within natural areas, often close to or within nature reserve boundaries (e.g. Duiwenhoks, Gatboskloof and Noukrans rivers). At these sites, there was generally a diverse mix of different macro-invertebrate taxa, of both tolerant and more sensitive

species (see tables under section 4 above). The species collected represented individuals from the very sensitive stonefly family Notonemouridae which scores 14 out of 15, down to the very tolerant true fly family Chironomidae, which scores two out of 15 (e.g. the Kruis River). Several of the rivers also housed taxa that are endemic to the South Western Cape rivers. These taxa, which consists of one mayfly family and some cased caddisfly families, are generally also very sensitive to pollution, with SASS scores ranging between 11 and 13 out of 15. These endemics included species of the mayfly family Teloganodidae (upper Huis, the upper Duiwenhoks, the Noukrans, the Kruis, upper Grootvadersbosch and the Gatboskloof rivers) and the cased caddisfly families Barbarochthonidae (Noukrans and upper Duiwenhoks), and Sericostomatidae (upper Duiwenhoks and the Noukrans rivers). The highest number of different taxa collected in the upper zones were from the uppermost Tradouw River site in the pass (21) and the upper Grootvadersbosch River (20). It should be noted here that these sites were sampled at different times of the year and more than a year apart, i.e. during November 2018 and February 2020 respectively.

The sites on the lower reaches of the rivers sampled, also generally had a rather diverse assemblage of different invertebrate families (the lowest Number of Taxa value recorded was 10 for both the lower Huis and lower Duiwenhoks rivers). However, the invertebrate taxa collected here were mainly from lower scoring families, subsequently resulting in lower SASS scores. The highest number of taxa collected were from the lower reaches, where at the lower Tradouw River (19) and lower Grootvadersbosch River (20) sites. The variation in SASS scores, and ASPT, between these two sites was mainly due to a few more, higher scoring taxa being collected at the lower Tradouw River site (e.g. Heptageniidae, Leptophlebidae, Philopotamidae and Pisulidae).

According to the national Level 1 Ecoregion classification and spatial layers (Kleynhans et al. 2015), only the Huis River (upper and lower) sites fall fully into the Southern Folded Mountains ecoregion. Furthermore, nine of the other SASS sites fall into the Southern Coastal Belt ecoregion, while the tenth site, the uppermost Tradouw River site, falls on the boundary between the two ecoregions. Nonetheless, all 12 sites are located within this transitional area between the two ecoregions and were therefore assessed taking both into account. The position of the sites in relation to the river zone (upland vs lowland; Dallas 2007) was also considered, as there are structural instream habitat differences between these two zones. The health condition values, derived from the presence of the different macro-invertebrate assemblages and their scores for the sites of the rivers sampled are depicted in Figures 7 (Southern Coastal Belt) and 8 (Southern Folded Mountains) below. According to the SASS and ASPT scores, there was considerable variance regarding the river health between the different sites, especially in the upper zones (Figure 7a and 8a). When taking into account both ecoregions, the Noukrans, Gatboskloof and upper Grootvadersbosch River sites show the

highest health scores within the A (natural) and B (near natural, minor modifications) range. The upper Duiwenhoks River has a health category of a high C in the Southern Coastal Belt, but a B in the Southern Folded Mountains. As all of these sites fall more directly into the Southern Coastal Belt ecoregion, and therefore the results depicted in Figure 7(a) might be more applicable. The rivers with lower health scores per site for the Southern Coastal Belt ecoregions, included the upper Huis (D) and Tradouw (D), as well as the Kruis (high D) and Tradouw River site upstream of the waterfall in the pass. Here, the latter site showed the most modified river health condition (category E). For these sites, the upper Huis River site falls more directly into the Southern Folded Mountain ecoregion, where it has a high D health category. Here the D category indicates a system that has been substantially impaired (water quality and/or habitat diversity), with SASS variables being much lower than expected and an almost total loss of the sensitive taxa (Dallas, 2007). The location of the upper Tradouw River is in the Tradouw pass, downstream of the lower Huis River site, which classifies it as falling into the rejuvenated zone, which in turn falls into the upland zone due to having similar biological characteristics (Dallas, 2007). The lower site on the Huis River was found to be in a C category at the time of sampling (Figure 7b).

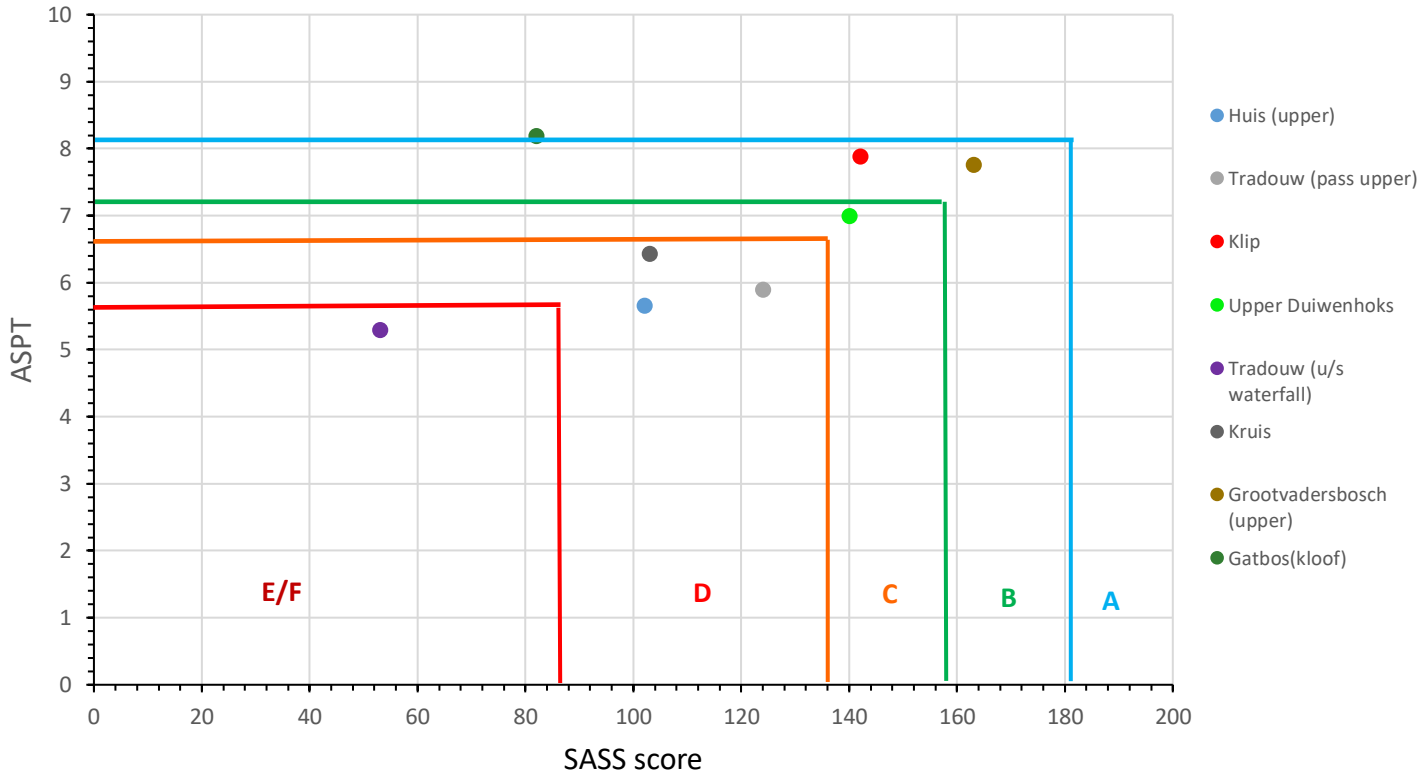

Figure 7 (a): The SASS scores and ASPT values at the eight sites, located in the mountain stream and upper foothill zones of the rivers sampled so far during the Grootvadersbosch Conservancy Area Freshwater survey. The different coloured circles depict the river sites. The coloured biological bands represent the changes in health condition relevant to the upper Southern Coastal Belt Level 1 Ecoregion (taken from Dallas 2007).

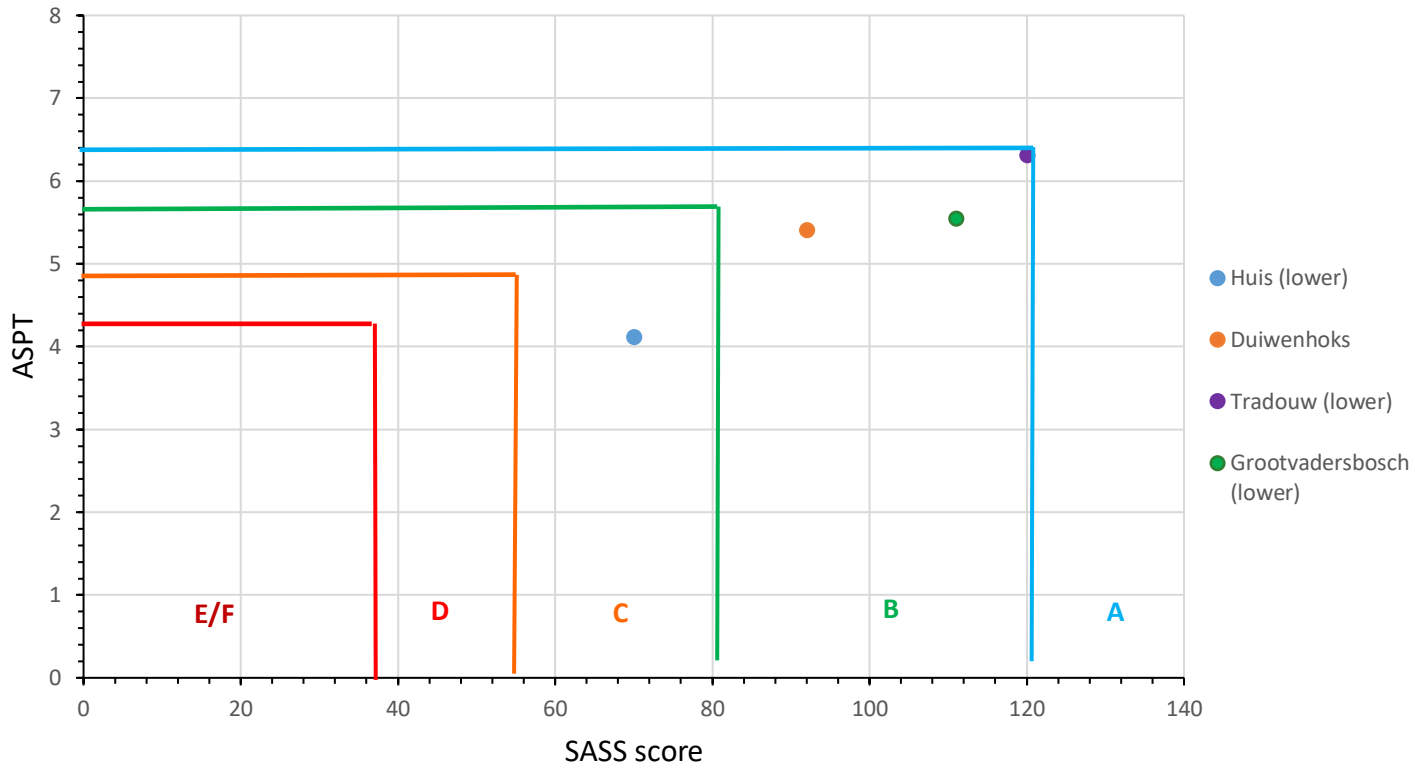

Figure 7 (b) The SASS scores and ASPT values at the four sites, located in the lower foothill and lower zones of the rivers that were sampled so far during the Grootvadersbosch Conservancy Area Freshwater survey. The different coloured circles depict the river sites. The coloured biological bands represent the changes in health condition relevant to the lower Southern Coastal Belt Level 1 Ecoregion (taken from Dallas 2007).

According to these results, the sites located in the lower zones had healthier river condition values at the time of sampling (Figure 7b and 8b). For this zone, the lowest condition category was found at the lower Huis River site (C, Southern Coastal Belt; low B Southern Folded Mountains). For this site, the Southern Folded Mountain results might be more applicable. The lower site on the Tradouw River was found to have the highest river health condition (A/B, Southern Coastal Belt; A, Southern Folded Mountains). The lower Duiwenhoks and Grootvadersbosch rivers were also found to have high health categories in both ecoregions, but as both of these sites fall more directly into the Southern Coastal Belt ecoregion, the B category might be more applicable (Figure 7b and 8b).

There is some variation with regard to impacts at the river sites sampled. It varies from the impacts of agriculture (Huis, Grootvadersbosch, Duiwenhoks and Tradouw) to urban impacts (Barrydale town) and those associated with water abstraction (weirs in the upper Huis River and the Noukrans River). This variance could affect several chemical characteristics of water, like the pH, conductivity and water temperature, which in turn could affect the presence of especially the more sensitive macro-invertebrate species. Another impact on these variables would be the effects during and after a fire, which was relevant to some of the sites (e.g. Tradouw River in the pass; upper Duiwenhoks River in Saagkuilskloof).

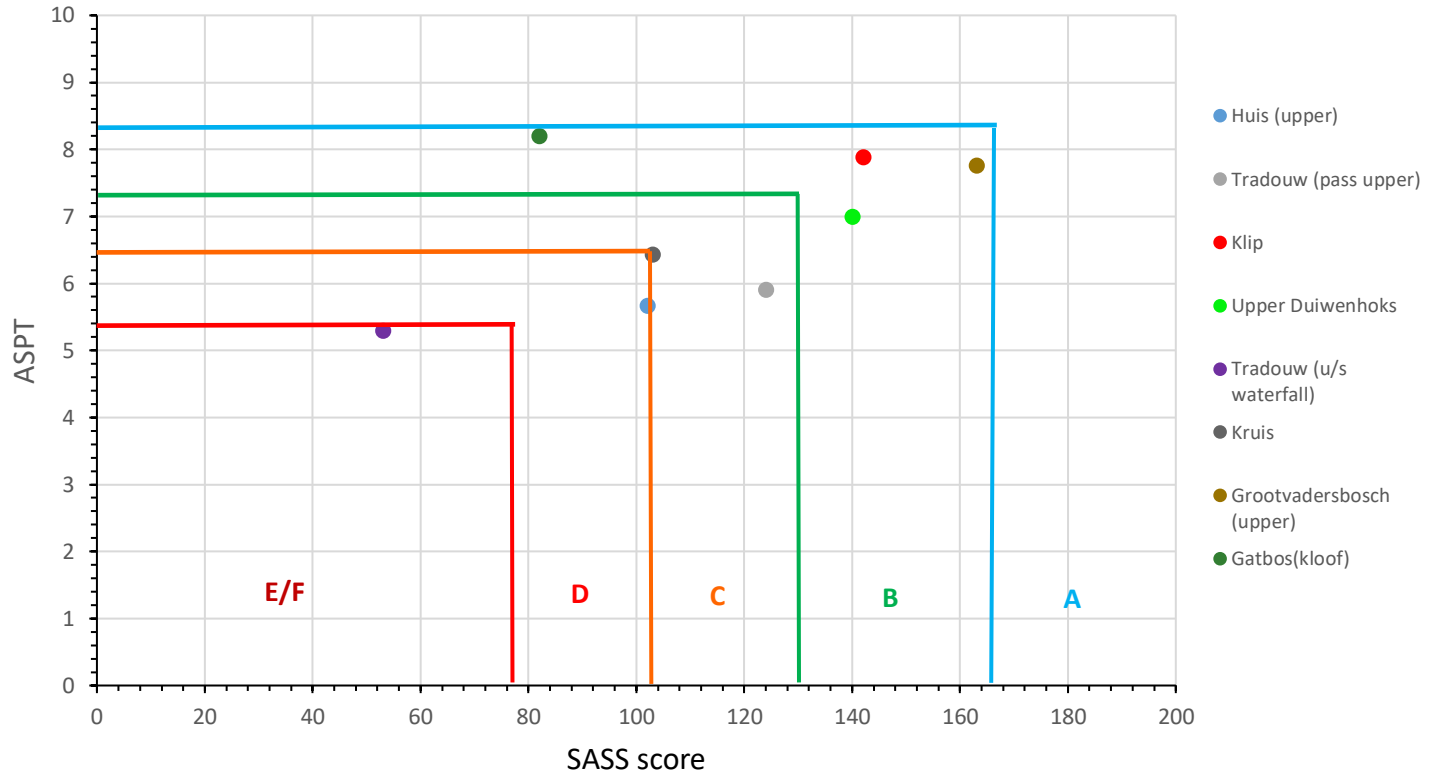

Figure 8 (a). The SASS scores and ASPT values at the eight sites located in the mountain stream and upper zones of the rivers that were sampled so far during the Grootvadersbosch Conservancy Area Freshwater survey. The different coloured circles depict the river sites. The coloured biological bands represent the changes in health condition relevant to the upper Southern Folded Mountains Ecoregion Level 1 (taken from Dallas 2007).

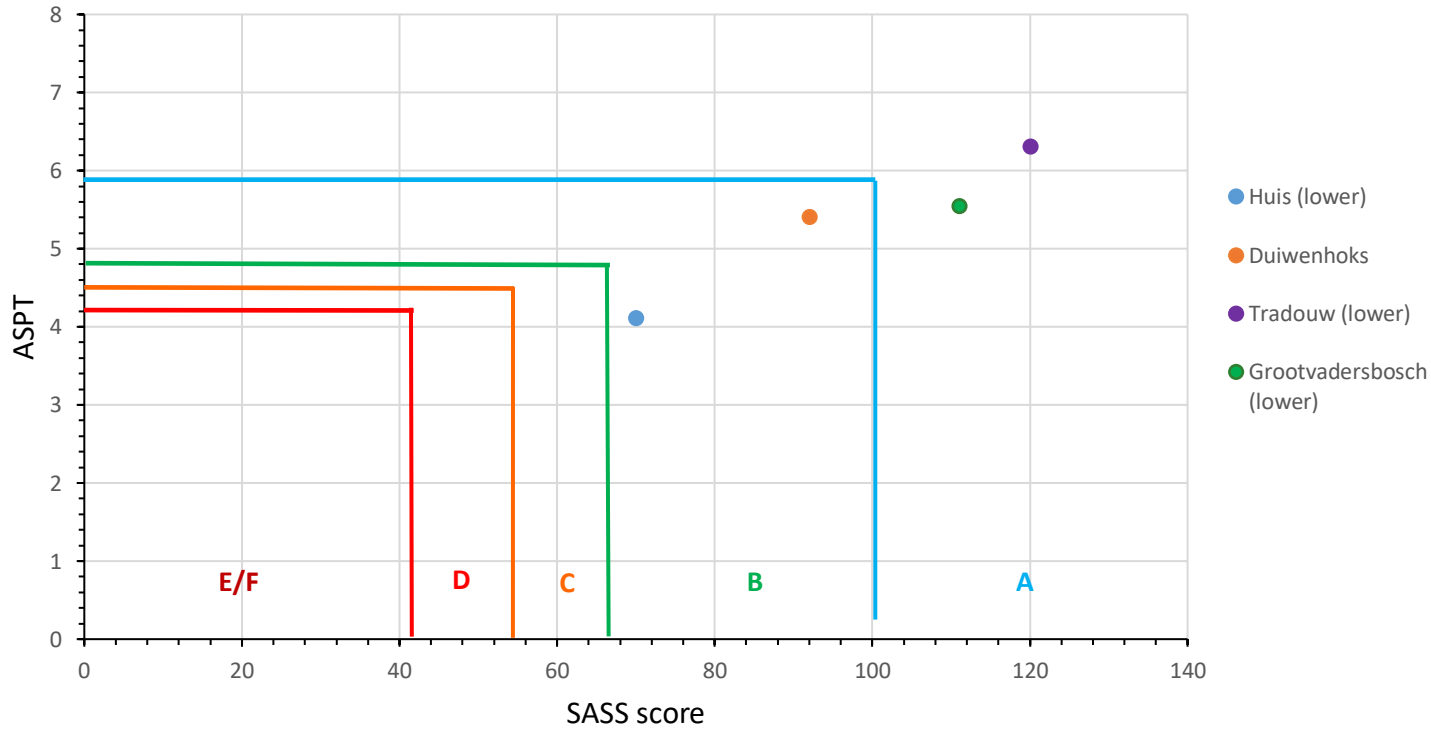

Figure 8 (b). The SASS scores and ASPT values at the four sites located in the lower foothill and lower zones of the rivers that were sampled so far during the Grootvadersbosch Conservancy Area Freshwater survey. The different coloured circles depict the river sites. The coloured biological bands represent the changes in health condition relevant to the lower Southern Folded Mountains Ecoregion Level 1 (taken from Dallas 2007).

The pH values were more acidic at all 12 sites. The highest value recorded was closer to being basic and was collected at the uppermost Tradouw River site (pH 6.65) in November 2018 (see Table 4.1.3 above). River flow was also high at this time in Tradouw pass. The two lower Tradouw River sites sampled in February 2020, both had pH values below 6 at the time of sampling, with the lowest Tradouw River sites having the most acidic pH (4.44) of the three sites. When comparing the other rivers with upper and lower sites (i.e. Duiwenhoks and Grootvadersbosch rivers), the trend was as expected. The upper sites had lower pH values than the lower sites, although the significance of this variation will need more in depth monitoring. For these two river systems, the Duiwenhoks River sites showed a slight difference in pH (upper pH 5.63; lower pH 5.72). These sites were sampled on different days (upper on 7 December 2018; lower on 6 December 2018), but moderate flow levels were observed at both. A more considerable difference was recorded at the upper and lower sites on the Grootvadersbosch River. These sites were sampled on the same day (26 February 2020), with the upper site value recorded at a more acidic pH of 4.3, while the lower site had a pH of 6.06. The lowest pH value recorded, was on the Kruis River (pH 3.61), with the Gatboskloof River water coming in at a close second with a pH of 4.03 in February 2020.

These patterns observed in the pH data are also evident in the conductivity values collected at these sites. For example, at the Huis River sites, down to the uppermost Tradouw River site, the recorded conductivity values increased from 0.0596 mS/m at the upper Huis River, to 0.184 mS/m at the lower Huis River and 0.237 mS/m at the Tradouw River site. The conductivity values recorded at the upper and lower Grootvadersbosch River sites, where it increased from 0.0805 mS/m at the upper site, to 0.129 mS/m at the lower site. It was the opposite for the Duiwenhoks River sites however, where the upper site had a slightly higher conductivity value (0.1078 mS/m) than the lower site (0.085 mS/m). Conductivity values recorded at the other sites were generally low, with values below 0.086 mS/m (see Tables in section 4). The lowest conductivity value recorded during the two surveys was on the upper Huis River in November 2018, while the Kruis River (0.0684 mS/m) had the lowest conductivity value recorded during the February 2020 survey.

The water temperatures recorded at the sites varied from a lowest of 18 °C (at the upper Grootvadersbosch River) to a highest value of 24 °C (at the lower Duiwenhoks River). In general, the upper sites had lower water temperatures than the lower sites at the times of sampling (e.g. Grootvadersbosch upper 18 °C vs lower Grootvadersbosch 23.1°C; see Tables 4.2.5 and 4.2.6 above). Variation of temperature is also known to influence the availability of dissolved oxygen (DO; mg/L) within water, especially at the water surface. For these two surveys, there was no clear or significant relationship between water temperature and DO. For example, the site where the lowest water temperature was recorded (upper Grootvadersbosch) only had the fourth highest recorded DO value (11.3 mg/L) at the time of sampling. The highest DO value was recorded

at the Tradouw River site upstream of the waterfall (12.1 mg/L). River flow was high and strong during sampling and the site was located in a riffle area between boulder cascades, which resulted in a lot of bubbling water. On the other hand, the DO reading at the lower Duiwenhoks River, which had the highest recorded temperature, also had the lowest DO value recorded (i.e. 6.2 mg/L). In order to get any clear patterns, each site should be considered separately and data should be recorded daily using a fixed meter. The SASS 5 data collected here provides only a snapshot of the water quality and biotope/habitat availability at each site of this baseline survey. Seasonal, more in depth invertebrate surveys are needed to get a complete picture of the species present and community structure and to determine the effects of certain impacts (as discussed in Barber-James and Pereira-da-Conceicoa, 2016). Additionally, the initial baseline survey only allows for very basic analyses to be done on the data, and patterns of seasonal, temporal and impact effect variance will only be picked up more clearly with long term monitoring of selected sites.

Regardless of these issues, the snapshot provided by the SASS 5 sampling done here is still very useful. Not only does it provide some information on water quality at a specific time, it also gives an indication of instream habitat availability, depicted through invertebrate taxon diversity and biotopes present. These initial SASS results can serve as a precursor to potential detailed studies on specifically the benthic macroinvertebrates, in association with indigenous and alien fish species distributions (see for example Bellingan *et al.*, 2015).

Due to the nature of this survey, many of the sites were located within reaches of the rivers where the riparian zones were still mostly intact. Here the greatest impacts were generally associated with the presence of invasive alien trees (e.g. black wattle, *A. mearnsii*) and the presence of abstraction weirs (e.g. Noukrans River). There were several exceptions to this pattern however, specifically for those sites located on private property and in the lower foothills, were invasive alien trees and local land use activities have resulted in the modification of riparian zones (e.g. amongst others, the lower Duiwenhoks, Tradouw, Huis and lower Grootvadersbosch rivers). The impacts associated with invasive alien species and land use activities, include the destabilisation of the river banks, as well as altering the natural shading pattern of the instream habitat (e.g. closed vs. open canopy). Some of the sites had naturally closed canopies (e.g., the Palmiet dominated systems such as the Duiwenhoks and Gatboskloof rivers) with mainly lower growing shrubs and trees, which resulted in natural levels of leaf. Other rivers had partially open canopies, with mostly indigenous higher level trees (e.g. the Noukrans, upper Duiwenhoks and Kruis rivers). The banks of these rivers were generally still stable, despite the effect of the fire in some of the catchments during September/October 2018. Altered shading patterns and other impacts associated with the presence of invasive alien trees and land use practices, were more prevalent at the lower lying sites, such as the lower

Grootvadersbosch and lower Duiwenhoks River systems. Some river sites were also impacted by weirs in the instream environment. These rivers include the Noukrans and upper and lower Huis rivers which have historical diversion weirs resulting in inundation directly upstream of the weirs. These instream structures in general also blocks most, if not all, of the water from flowing further downstream, often leading to dried out riverbeds downstream. This is known to be the case for the upper Huis River, especially during the dry, hot summer months. Other instream impacts noted, were the presence of roads and road crossings which also impact the hydrological flow patterns of the river system (e.g. lower Duiwenhoks rivers).

These riparian zone and instream impact patterns, where also somewhat reflected in the SASS and physico-chemical results, in that the lower lying impacted sites, generally had the lower SASS and ASPT scores leading to a C or D (moderately to largely modified) ecosystem health categorisation. For the most part though, the river sites in more natural areas all housed a variety of sensitive, high scoring macro-invertebrate taxa and were found to be in at least a good (B) or good to natural (AB) condition, as would be expected. Even for the sites that had weirs on them. Many of the sites also housed known SWC endemic insect families, which were also expected due to the more acidic nature and naturally tannin-stained waters of the rivers this area. Additionally, the instream habitats were still diverse and largely intact, with the river water also not being impaired with regards to quality. Algae was present to varying degrees throughout the river systems, mostly at naturally expected levels, in isolated clumps were it was noticed. The exceptions to this was in the lower Huis River, were the algal cover was found to be substantial as a result of nitrification of the system. Here the water quality is impaired and impacts include over-abstraction of water, leading to lower water levels and increased water temperatures, as well as those associated with run off from the upstream small scale farming practices and the storm water entering the river as it travels through the town of Barrydale. This particular site is also located just upstream of a weir and next to the Barrydale winery, but it still provides habitat for a population of the Barrydale redfin.

In conclusion, despite this survey only providing the baseline survey data for this Grootvadersbosch Conservancy freshwater survey, some patterns, especially in terms of SASS5 results, have already become apparent. The most significant impacts on the rivers included in this survey have been identified, and include the presence of alien invasive tree and to some degree fish species, the effects of the recent drought, complete river diversion and over abstraction of water for domestic and agricultural use. In this way, the upper reaches of the river located within the more natural areas, could allow space for dispersal of aquatic macroinvertebrates under the continued pressures associated with land use practices (Petersen et al. 2004) and climate change. Ideally, seasonal monitoring of strategically selected sites should be implemented in the long term, especially related to those rivers that support indigenous fish species. Continued monitoring is

also considered important here because the GVB Conservancy area includes a part of the mountain catchment which forms part of one of the Strategic Water Source Areas for the Western Cape Province [WWF 2013 (a) and (b)].

6. GASPP project summary

This project is a significant contribution towards the objectives outlines in the Grootvadersbosch Aquatic Species Protection Project. The project has two key outcomes:

1. To ensure and initiate long-term monitoring of key data (flow, fish sampling, water quality, SASS, etc.) of the Grootvadersbosch Conservancy Rivers (Tradouw River and smaller tributaries, Grootvadersbos River and Buffeljags River); and
2. To increase stakeholder awareness on the importance of freshwater ecosystems in the project area to sustain water for environmental, agricultural and municipal use.

This project is part of a larger vision that eventually aims to halt the decline of freshwater fish species in the Grootvadersbosch Conservancy Rivers and facilitate recovery through securing critical habitat and the reduction of anthropogenic impacts on aquatic species. In time, this will hopefully lead to downgrading of indicator species from Critically Endangered status. Part of the larger vision and not in the scope of this project, will be the implementation of key conservation actions arising from formalised stakeholder engagement and partnerships supported by the monitoring data collected through this project.

A key objective of the project is to build capacity within the Grootvadersbosch Conservancy to be able to continue to monitor the rivers within the Conservancy without extensive external support. A key part of that process is to expose staff to the required techniques for accurate sampling of data. The team is already showing adequate experience in fish sampling and has obtained experience in SASS. We have investigated the requirements for SASS accreditation and have been advised that in-field experience prior to undertaking the costly training is recommended. These in-field trips therefore make a significant contribution towards the goal of training staff for SASS accreditation.

The sites surveyed covered a wide range of important sites that give an excellent overview of the status of the rivers in the Conservancy. Unfortunately, due to bad weather, a few sites were not sampled. The team had then planned to revisit them a few weeks later but the COVID19 lock down prevented the proposed continuation of fieldwork and training. The main gap in sites in the main Grootvadersbosch River upstream and downstream of the town of Suurbraak. In addition, as discussed above, the analysis of fish distribution

outlined key questions that require further sampling to better understand fish distribution and impacts within the system. The sampling of these sites will be planned when lockdown restrictions are eased, and travel is again possible. As the short-term future remains uncertain amidst the pandemic, it is a possibility that the continuation of sampling will only be possible next summer (2020/2021).

6.1 Biological data collection summary

The data presented was collected in December 2018 and February-March 2020. Data collected thus far will aid in meeting objectives of the GASPP project, but also serve the following purposes beyond the GASPP project:

(a) fish distribution and SASS5 data will provide a baseline for the Langeberg Nature Reserve Complex Integrated Managed Plan currently being developed by CapeNature.

(b) SASS5 data that was collected in the Tradouw catchment will provide baseline data for inclusion into the draft Barrydale redfin BMP-S monitoring protocols for initiating long term SASS5 monitoring.

(c) DNA samples and voucher specimens will be lodged into the national fish collection housed at the South African Institute for Aquatic Biodiversity (SAIAB) where it will be accessible to other researchers, most importantly to those currently working on the taxonomic revision for *G. zebratus* and *S. capensis*.

(d) Fish population size and distribution data, as well as new distribution records, will be incorporated in future IUCN Red List Assessments.

6.2 Human capital development

The survey was planned and coordinated by CapeNature staff with the assistance from GVB Conservancy staff. During fieldwork, the following training and personal development opportunities were provided:

(a) Practical experience in biotope identification and the collection of SASS5 invertebrate samples.

(b) Training in the identification of aquatic invertebrates using field guides and interpretation and completion of SASS field forms.

(c) Practical experience in conducting fish sampling using different gear types.

(d) Training in the identification of freshwater fish using field guides, collecting biological measurements and completing datasheets.

(e) Practical experience in collecting basic water quality data.

(e) Collection and preservation of DNA and voucher specimens for incorporation in the National Fish Collection.

(f) Experience in the analysis and write up of field data

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