Supplementary Material

# Supplementary Figures and Tables

## Supplementary Tables

**Supplementary Table 1. Fish communities present in the sampled river basins adapted from Ellender et al. (2011) and Skelton (2001).**

|  |  |  |
| --- | --- | --- |
| Species | Swartkops | Kouga |
| Native |   |   |
| African Longfin Eel (*Anguilla mossambica*) | ✓ | ✓ |
| Giant Mottled Eel (*Anguilla marmorata*) | ✓ | ✓ |
| Eastern Cape Redfn (*Pseudobarbus afer*) | ✓ |  |
| Gamtoos Redfin (*Pseudobarbus swartzi*) |  | ✓ |
| Goldie Barb (*Enteromius pallidus*) | ✓ | ✓ |
| Cape Kurper (*Sandelia capensis*) | ✓ | ✓ |
| River Goby (*Glossogobius callidus*) | ✓ | ✓ |
| Non-native |   |   |
| Common Carp (*Cyprinus carpio*) | ✓ | ✓ |
| African Sharptooth Catfish (*Clarias gariepinus*) | ✓ | ✓ |
| Largemouth Bass (Micropterus salmoides) | ✓ | ✓ |
| Smallmouth Bass *(Micropterus dolomieu)* | ✓ | ✓ |
| Spotted Bass (*Micropterus punctulatus*) | ✓ |  |
| Banded Tilapia (*Tilapia sparmanii*) | ✓ | ✓ |

**Supplementary table 2. Species-specific primers (*cytb*) designed for this study**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Name** | **Primer Sequence** | **bp** | **Tm (°C)** | **GC %** | **Product Length** |
| Redfin\_184f | GCATTTTCATCAGTAGCCCAT | 21 | 56.63 | 42.86 | 132 bp |
| Redfin\_315r | GCCGTAGTATAGTCCTCGAG | 20 | 56.46 | 55.00 |
| Kurper\_226f | AGGAGAAGAACTACGCCTG | 19 | 56.17 | 52.63 | 137 bp |
| Kurper\_367r | GGCTGACTTATCCGAAATCTC | 21 | 56.22 | 47.62 |

**Supplementary table 3. Detection and non-detection for underwater cameras, snorkel surveys, and eDNA – qPCR data for biological and technical PCR replicates.**

|  |  |  |
| --- | --- | --- |
| **Species** | ***Pseudobarbus swartzi*** | ***Sandelia capensis*** |
| **Site** | **Ca** | **Sn** | **eDNA B1** | **eDNA B2** | **eDNA B3** | **Mean Cq** | **Ca** | **Sn** | **eDNA B1** | **eDNA B2** | **eDNA B3** | **Mean Cq** |
| BK1 | 1 | 1 | - | - | - | - | 1 | 1 | - | - | - | - |
| BK10 | 1 | 1 | - | - | - | - | 1 | 1 | - | - | - | - |
| BK11 | 1 | 1 | - | - | - | - | 0 | 0 | - | - | - | - |
| BK12 | 1 | 1 | 2/3 | 3/3 | 0/3 | 32.86 | 0 | 0 | 0/3 | 0/3 | 0/3 | - |
| BK14 | 1 | 1 | - | - | - | - | 0 | 0 | - | - | - | - |
| BK16 | 1 | 1 | - | - | - | - | 1 | 1 | - | - | - | - |
| BK17 | 1 | 1 | - | - | - | - | 1 | 1 | - | - | - | - |
| BK18 | 1 | 1 | - | - | - | - | 1 | 1 | - | - | - | - |
| BK2 | 1 | 1 | - | - | - | - | 1 | 1 | - | - | - | - |
| BK23 | 1 | 1 | - | - | - | - | 1 | 1 | - | - | - | - |
| BK27 | 1 | 1 | 2/3 | 2/3 | 3/3 | 33.56 | 1 | 1 | 1/3 | 3/3 | 1/3 | 33.1 |
| BK3 | 1 | 1 | 2/3 | - | 1/3 | 33.01 | 1 | 1 | 2/3 | - | 1/3 | 34.05 |
| BK31 | 1 | 1 | - | - | - | - | 1 | 1 | - | - | - | - |
| BK32 | 1 | 1 | 0/3 | 0/3 | 3/3 | 34.51 | 1 | 1 | 0/3 | 1/3 | 2/3 | 34.76 |
| BKConf | 1 | 1 | - | - | - | - | 1 | 0 | - | - | - | - |
| BKe | 1 | 0 | - | - | - | - | 0 | 0 | - | - | - | - |
| BKNF | 0 | 0 | - | - | - | - | 0 | 0 | - | - | - | - |
| Bos1 | 0 | 0 | - | - | - | - | 0 | 0 | - | - | - | - |
| Bos10 | 0 | 1 | - | - | - | - | 0 | 0 | - | - | - | - |
| Bos11 | 1 | 1 | - | - | - | - | 1 | 1 | - | - | - | - |
| Bos12 | 1 | 1 | - | - | - | - | 1 | 0 | - | - | - | - |
| Bos13 | 1 | 1 | - | - | - | - | 1 | 0 | - | - | - | - |
| Bos14 | 1 | 1 | 3/3 | 3/3 | 3/3 | 29.63 | 0 | 0 | 2/3 | 0/3 | 0/3 | 34.4 |
| Bos15 | 1 | 1 | - | - | - | - | 0 | 0 | - | - | - | - |
| Bos16 | 1 | 1 | - | - | - | - | 0 | 0 | - | - | - | - |
| Bos17 | 1 | 1 | 3/3 | 3/3 | 3/3 | 28.02 | 0 | 0 | 0/3 | 0/3 | 0/3 | - |
| Bos18 | 0 | 0 | - | - | - | - | 0 | 0 | - | - | - | - |
| Bos19 | 0 | 0 | - | - | - | - | 0 | 0 | - | - | - | - |
| Bos2 | 0 | 0 | - | - | - | - | 1 | 1 | - | - | - | - |
| Bos20 | 0 | 0 | - | - | - | - | 0 | 0 | - | - | - | - |
| Bos21 | 0 | 0 | - | - | - | - | 0 | 0 | - | - | - | - |
| Bos22 | 0 | 0 | - | - | - | - | 0 | 0 | - | - | - | - |
| Bos23 | 0 | 0 | - | - | - | - | 0 | 0 | - | - | - | - |
| Bos3 | 1 | 1 | 3/3 | 3/3 | 3/3 | 32.19 | 1 | 1 | 3/3 | 3/3 | 3/3 | 31.32 |
| Bos4 | 1 | 1 | - | - | - | - | 1 | 1 | - | - | - | - |
| Bos5 | 0 | 0 | 0/3 | 0/3 | 0/3 | - | 0 | 0 | 0/3 | 0/3 | 0/3 | - |
| Bos6 | 0 | 0 | - | - | - | - | 0 | 1 | - | - | - | - |
| Bos7 | 1 | 1 | - | - | - | - | 1 | 1 | - | - | - | - |
| Bos8 | 1 | 1 | 3/3 | 3/3 | 3/3 | 32.77 | 1 | 1 | 3/3 | 1/3 | 2/3 | 32.77 |
| Bos9 | 1 | 1 | 3/3 | 0/3 | 3/3 | 29.73 | 1 | 1 | 3/3 | 1/3 | 2/3 | 31.8 |
| FK1.2 | 1 | 1 | - | - | - | - | 0 | 0 | - | - | - | - |
| FK10 | 0 | 0 | - | - | - | - | 0 | 0 | - | - | - | - |
| FK21 | 1 | 1 | - | - | - | - | 1 | 1 | - | - | - | - |
| FK22 | 1 | 1 | 0/3 | 2/3 | 2/3 | 35.55 | 1 | 1 | 2/3 | 2/3 | 0/3 | 33.7 |
| FK23 | 1 | 1 | - | - | - | - | 1 | 1 | - | - | - | - |
| FK24 | 1 | 1 | - | - | - | - | 1 | 1 | - | - | - | - |
| FK3 | 1 | 1 | - | - | - | - | 0 | 1 | - | - | - | - |
| FK4 | 1 | 1 | - | - | - | - | 1 | 1 | - | - | - | - |
| FK5 | 1 | 1 | 0/3 | 0/3 | 0/3 | - | 1 | 1 | 0/3 | 0/3 | 0/3 | - |
| FK8 | 1 | 1 | - | - | - | - | 0 | 0 | - | - | - | - |
| FK9 | 1 | 1 | - | - | - | - | 0 | 0 | - | - | - | - |
| Fkbwf | 1 | 1 | 0/3 | 3/3 | 0/3 | 30.37 | 0 | 0 | 0/3 | 0/3 | 0/3 | - |
| FKwf | 0 | 0 | 0/3 | 0/3 | 0/3 | - | 0 | 0 | 0/3 | 0/3 | 0/3 | - |
| K1 | 0 | 0 | 0/3 | 0/3 | 0/3 | - | 0 | 0 | 0/3 | 0/3 | 0/3 | - |
| K2 | 0 | 0 | - | - | - | - | 0 | 0 | - | - | - | - |
| K3 | 0 | 0 | 0/3 | 0/3 | 0/3 | - | 0 | 0 | 0/3 | 0/3 | 0/3 | - |
| K4 | 0 | 0 | 0/3 | 0/3 | 0/3 | - | 0 | 0 | 0/3 | 0/3 | 0/3 | - |
| WK | 1 | 1 | - | - | - | - | 1 | 1 | - | - | - | - |
| WK2 | 1 | 1 | - | - | - | - | 0 | 0 | - | - | - | - |
| WK21 | 1 | 1 | 0/3 | 0/3 | 3/3 | 30.37 | 0 | 0 | 0/3 | 0/3 | 0/3 | - |
| WK22 | 1 | 1 | - | - | - | - | 0 | 0 | - | - | - | - |
| WK23 | 1 | 0 | - | - | - | - | 1 | 1 | - | - | - | - |
| WK24 | 1 | 1 | 0/3 | 0/3 | 1/3 | 36.13 | 1 | 1 | 2/3 | 0/3 | 0/3 | 35.83 |
| WK3 | 1 | 1 | 0/3 | 2/3 | 1/3 | 32.15 | 1 | 1 | 0/3 | 2/3 | 0/3 | 33.2 |
| WK4 | 1 | 0 | - | - | - | - | 0 | 0 | - | - | - | - |
| WK5 | 0 | 0 | - | - | - | - | 1 | 0 | - | - | - | - |
| WKWF | 1 | 1 | 0/3 | 0/3 | 0/3 | - | 0 | 0 | 0/3 | 0/3 | 0/3 | - |

Ca: detection with Camera survey; Sn: detection with Snorkel survey; eDNA B1-3: proportion of technical replicates returning a “hit” in each eDNA biological replicate; Mean Cq: qPCR quantification cycle; -:no survey with method

## Supplementary Figures

**Supplementary Figure 1.** Standard curve generated with serially diluted *Pseudobarbus swartzi* and *Sandelia capensis* genomic DNA. Dilution gradient represents genomic DNA concentrations from 10 ng/μL to 1 × 10-6 ng/μL determined using a Qubit Fluorometer. The LOD was determined to be 1 × 10-4 ng/μL for *Pseudobarbus swartzi* and 1 × 10-5 ng/μL for *Sandelia capensis.*