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Short note

Preliminary evaluation of non-native rainbow trout (*Oncorhynchus mykiss*) impact on the Cederberg ghost frog (*Heleophryne depressa*) in South Africa's Cape Fold Ecoregion

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We evaluated the impact of non-native rainbow trout *Oncorhynchus mykiss* on a population of endemic Cedarberg ghost frog *Heleophryne depressa* in the upper Krom River (Olifants-Doring River Catchment, Cape Fold Ecoregion). We compared *H. depressa* abundance (using kick-sampling and underwater video analysis) and environmental conditions between sites above and below a waterfall that marks the upper distribution limit of *O. mykiss*. *Heleophryne depressa* abundance was significantly greater above the waterfall than that below it, and, because there was no significant difference in measured environmental variables, *O. mykiss* presence is identified as the most likely explanation for the observed decrease in *H. depressa* abundance.

Keywords: Amphibian, endemic species, freshwater fish, kick-sampling, Olifants-Doring River Catchment, underwater video analysis, waterfall barrier

Amphibians are the most threatened class of vertebrate in the world (Foden et al. 2013). An estimated 122 amphibian species have become extinct since 1980 (Gascon et al. 2007), and of the remaining ~6 000 extant species assessed by the International Union for Conservation of Nature IUCN (2017), 32% of species are threatened and 42% are in decline. Principal threats to amphibians include habitat fragmentation and loss, hydraulic alteration, pollution, pathogenic infection, increased ultraviolet radiation and non-native species impacts (Strayer and Dudgeon 2010).

Amphibian populations have been detrimentally affected by invasions by non-native plants (Gascon et al. 2007), crayfish (Ficetola et al. 2011) and other amphibians (Kats and Ferrer 2003), but impacts by non-native fish have been particularly extensive and well-studied (Kats and Ferrer 2003). A wealth of correlative studies show strong negative impacts of non-native fish on native amphibian populations (see review by Kats and Ferrer 2003), whereas experimental manipulations have shown that predation and competition by non-native fish can influence amphibian abundance, behaviour, morphology and physiology (see review by Nyström et al. 2001). In contrast, impacts of non-native fishes on amphibians in South Africa have not been extensively studied. To our knowledge, the only published study from South Africa demonstrated that declines in the abundance the Natal cascade frog *Hadromophryne natalensis* (Hewitt, 1913) were associated with the presence of non-native rainbow *Oncorhynchus mykiss* (Walbaum, 1792) and brown trout *Salmo trutta* Linnaeus, 1758 below waterfall barriers in two headwater streams in KwaZulu-Natal (Karssing et al. 2012).

Despite widespread non-native fish invasions and documented ecological impacts (Ellender and Weyl 2014), no studies have yet investigated non-native fish impacts on amphibians in South Africa's Cape Fold Ecoregion (CFE), a global freshwater biodiversity hotspot (de Moor and Day 2013). The CFE is home to approximately 40 species of endemic amphibians (Mokhatla et al. 2015), including six members of the 'ghost frogs', genus *Heleophryne*, of which two species *Heleophryne hewitti* Boycott, 1988 and *Heleophryne rosei* Hewitt, 1925 have been evaluated as Endangered and Critically Endangered, respectively (IUCN 2017). Headwater streams in the CFE have been extensively invaded by the non-native salmonid, *O. mykiss* and, although negative impacts on native fish (Shelton et al. 2015a) and aquatic invertebrates (Shelton et al. 2015b) have been documented, impacts on amphibian populations have not previously been quantified.

We evaluated the impact of *O. mykiss* on a population of endemic Cederberg ghost frog *Heleophryne depressa* FitzSimons, 1946 in the upper Krom River, a near pristine headwater stream in the Olifants-Doring River Catchment of the Cederberg Mountains in the northwestern corner of the CFE (Figure 1), to ascertain whether the inverse relationship between trout and amphibian abundance observed in KwaZulu-Natal (Karssing et al. 2012) was repeated in the CFE. *Heleophryne depressa* grows to 45 mm in length and requires swift-flowing, perennial, undisturbed headwater streams as breeding sites (du Preez and Carruthers 2009). Their tadpoles are well-adapted to such environments with flattened, streamlined bodies and large oral disks for sucking onto rocks and feeding on algae (du Preez and Carruthers 2009). The development of *H. depressa* tadpoles is slow and might take up to two years before metamorphosis is complete (du Preez and Carruthers 2009).

The Krom River originates in a mountainous seep at approximately 1 400 m asl, and flows in an easterly direction for about 25 km where it joins the Matjies River, which then feeds into the Doring River (Figure 1b). The area is comprised mostly of quartzitic Table Mountain Sandstone (Tankard et al. 1982) surrounded by Cederberg Sandstone Fynbos (Mucina and Rutherford 2011). The stream is narrow (generally <5 m wide), shallow (generally <1 m deep) and is comprised of chutes, pools, bedrock steps and cobble-bed riffles (Shelton et al. 2017). *Oncorhynchus mykiss* was first introduced to parts of the Olifants-Doring River Catchment as early as 1897 (de Moor and Bruton 1988) and it has been present in the Krom River for more than 60 years (Marr et al. 2012), with a
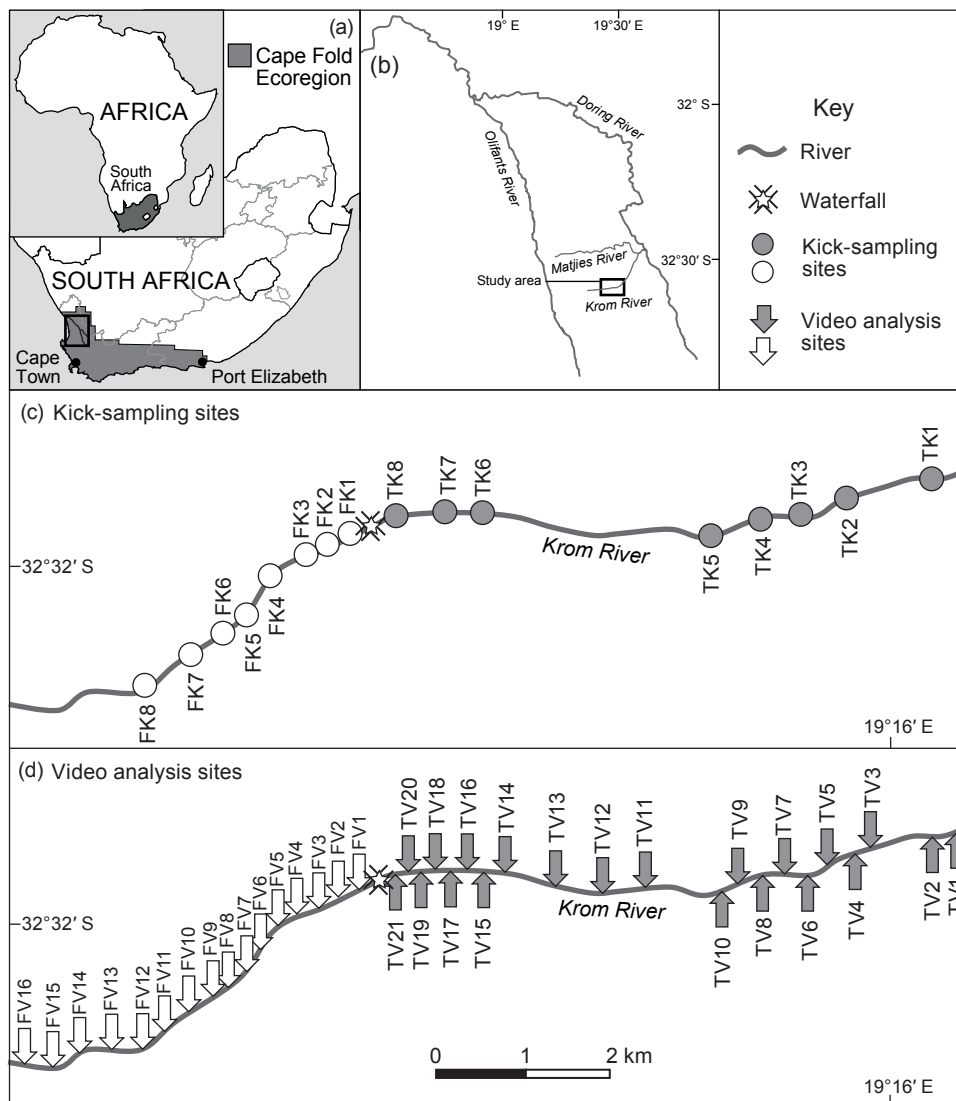

Figure 1: Location of sampling sites on the Krom River in the Olifants-Doring River Catchment in the Cape Fold Ecoregion. Panel (b) shows an enlargement of the grey rectangle in panel (a), and panels (c) and (d) show enlargements of the grey rectangle in Panel (b). Panel (c) shows the locations of the kick-sampling sites, and panel (d) the locations of the video analysis sites. Grey symbols represent sites below the waterfall (kick-sampling sites TK1-TK8, video analysis sites TV1-TV21) and white symbols represent sites above the waterfall (kick-sampling sites FK1–FK8, video analysis sites FV1-FV16)

5 m-high waterfall marking their upstream distribution limit (Shelton et al. 2017). The native Clanwilliam rock catfish *Austroglanis gilli* Barnard, 1943 is also present in the upper Krom River (Marr et al. 2012), but the upper limit of its distribution fell downstream of our study reach and *O. mykiss* was the only fish species detected in the study reach.

We compared the relative abundance of *H. depressa* between sites above the waterfall (fishless zone; sites denoted with F) and below it (trout zone; sites denoted with T) to infer whether *O. mykiss* had an influence on the *H. depressa* population. We concurrently evaluated selected habitat parameters to assess the potential influence of habitat variation on *H. depressa* abundance. Sixteen accessible, 20 m-long sites were selected from available stony, flowing habitats; eight above the waterfall (F, *O. mykiss* absent) and eight below it (T, *O. mykiss* present) (Figure 1c). *Heleophryne depressa* abundance was estimated using two methods; kick-sampling and video analysis. To our knowledge, using underwater video analysis to estimate tadpole relative abundance is novel to this study. The kick-sampling method (adapted from Belden et al. 2007) involved disturbing stones by kicking and netting tadpoles throughout the site for 5 min.

The video analysis (based on Ellender et al. 2012) used Go-Pro Hero 3+ cameras in underwater housings mounted on tripods weighted to the stream bed and set to record (1 080 p, 30 fps) for 20 min. Sites for the cameras included randomly-selected, accessible locations between the sixteen kick-sampling sites, and incorporated all available biotopes (Ellender et al. 2012). Sixteen sites were sampled above the waterfall using the video analysis and 21 sites were sampled below it (Figure 1d). The maximum number of tadpoles and trout in the field of view at the same time (MaxN, *sensu* Cappo et al. 2004) was estimated for each site over 15 min of footage, excluding a five-min acclimation period. The number of tadpoles recorded by each method was compared between sites above and below the waterfall using Mann–Whitney *U* tests, because the data did not meet the requirements for parametric analyses, even after transformation.

On completion of amphibian sampling, a set of environmental variables was measured at each kick-sampling site. Three width transects (at right angle to flow direction) were laid at equidistant points along the length of each 20 m length of the site for estimation of channel width, depth and substrate composition. Channel width was measured with a tape measure and depth measurements were taken at five equidistant points along each width transect using a calibrated depth rod. Mean width was estimated from the three width transects, and mean depth from the 15 depth measurements. Substrate type at each point was estimated visually using the substrate categories defined by Rowntree and Wadeson (2000), including bedrock, boulder, pebble, gravel and sand, and these data were used to estimate the proportional substrate composition at each site. Water physico-chemical parameters, including pH, electrical conductivity (μS cm^{-1}), total dissolved solids (ppm) and temperature (°C) were measured at a random location at each site using a Hanna water quality multi-meter (HI 9143).

All environmental variables were checked for normality using Shapiro–Wilk tests, and pH, electrical conductivity, temperature, total dissolved solids and mean width and depth were square root-transformed, whereas all substrate categories were arcsine-square-root transformed to even out their skewed distributions (Quinn and Keough 2002). A principal component analysis (PCA) ordination was used to summarize and visualize differences in environmental conditions between the sampling sites. One-way permutational multivariate analysis of variance (PERMANOVA) was used to assess whether or not measured environmental conditions differed significantly between the kick-sampling sites above and below the waterfall (Anderson et al. 2008), and a permutational analysis of multivariate dispersion test (PERMDISP), the main assumption of PERMANOVA (Anderson et al. 2008), used to test for a significant difference in dispersion between the two groups of sites.

In the PCA ordination (58% variation captured by PC axes 1 and 2), there was no obvious separation of the sites above the waterfall from the sites below it (Figure 2), and differences in environmental conditions between these two zones were not significant (one-way PERMANOVA; $F = 0.27$, $p = 0.717$). There was also no significant difference in sample dispersion of sites between the two zones (one-way PERMDISP; $F = 0.05$, $p = 0.788$).

The kick-sampling data indicated that *H. depressa* was present at all eight sites above the waterfall, but only at three of the sites below it. Mean relative abundance (number of tadpoles per site ± SE) above the waterfall (9.13 ± 4.29) was significantly higher than that below it (0.63 ± 0.92, Mann–Whitney *U* test; $U = 64$; $p = 0.001$). A χ^2 analysis of the kick-sampling showed a significant difference in *H. depressa* tadpole detection rates above and below the waterfall ($\chi^2 = 7.27$, $p > 0.007$). The video analysis shows a similar pattern, but no *H. depressa* were recorded below the waterfall using this method (and
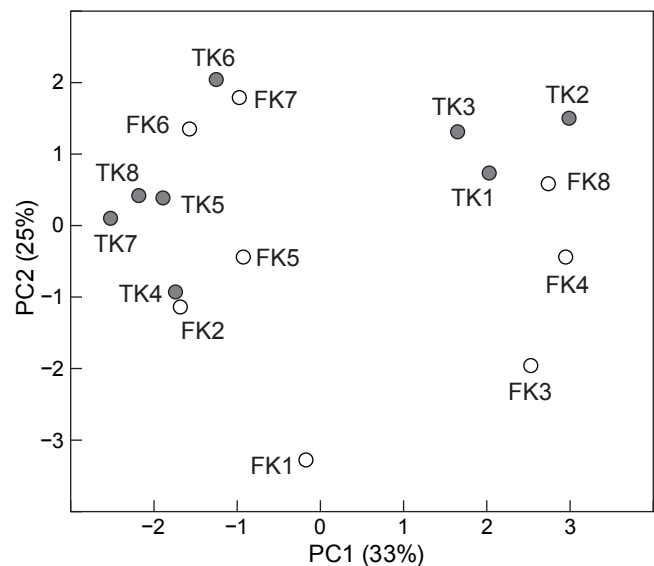

Figure 2: Principal components analysis ordination summarizing environmental conditions at kick-sampling sites above (white circles: FK1-8) and below (grey circles: TK1-8) the waterfall. PC 1 represents 33%, and PC 2 represents 25%, of the total variation in environmental conditions among sites

consequently no statistical analyses were conducted on abundance estimates derived from this method). *Heleophryne depressa* were recorded at seven of the 16 sites above the waterfall, and MaxN ranged from 1 to 5. A χ^2 analysis of the video analysis showed a significant difference in *H. depressa* tadpole relative abundance above and below the waterfall ($\chi^2 = 13.04$, $p < 0.001$). A χ^2 analysis showed a significant difference in the *H. depressa* tadpole detection rates between kick-sampling and video analysis ($\chi^2 = 91.45$, $p < 0.001$) with kick-sampling having a higher *H. depressa* relative abundance than video analysis. A video analysis confirmed that *O. mykiss* was present below the waterfall, but not above it. *Oncorhynchus mykiss* was recorded at eight of the 21 sites below the waterfall, and MaxN ranged from 1 to 2.

Given the lack of consistent difference in measured environmental variables between the sites above and below the waterfall, the presence of *O. mykiss* is implicated as the main factor responsible for significant decrease in *H. depressa* abundance below the waterfall. This finding is consistent with studies in South Africa (Karssing et al. 2012) and elsewhere (Kats and Ferrer 2003) documenting similar negative associations between trout and native amphibian populations. Given their predatory impact on native fishes in the CFE (e.g. Shelton et al. 2015a), and on amphibians elsewhere (e.g. Gillespie 2001; Barr and Babbitt 2002), trout predation is the most likely mechanism behind the lower abundance of *H. depressa* below the waterfall. Indeed, *O. mykiss* could have significant impacts on native biota even where their abundance is relatively low (Shelton et al. 2015a), as appears to be the case in the current study (Figure 3). The role of predation could be further investigated through analysis of trout diet in the wild (e.g. Remon et al. 2016), through the use of stable isotopes (Finlay and Vredenburg 2007) and through predation experiments (e.g. Gillespie 2001).

Other mechanisms could also contribute to the decreased abundance of *H. depressa* where *O. mykiss* is present. In addition to direct predation, non-native fish can also cause reduced metomorph size and rate (Nystrom et al. 2001), adjusted habitat use (Tyler et al. 1998), decrease in body size and decreases in weight (Tyler et al. 1998) and change the behaviour of both adults and tadpoles (Wilson et al. 2017). Moreover, chemical cues from predatory fish can cause adult amphibians to adjust preferred locations for depositing eggs (Wilson et al. 2017) and delayed or premature hatching occurs in tadpoles (Fraker et al. 2009).

Our data do not permit us to disentangle consumptive vs behavioural responses of *H. depressa* to *O. mykiss*, but are suggestive of *H. depressa* changing its behaviour in the presence of *O. mykiss*. Our video analysis method, which detected *H. depressa* at several of the sites above the waterfall, did not record any *H. depressa* below the waterfall (based on the deployment of 18 cameras capturing at total of more than 300 min of GoPro footage). That the kick-sampling recorded *H. depressa* at three of the eight study sites, suggests that, although present below the waterfall, *O. mykiss* might suppress *H. depressa* activity and/or result in increased shelter-seeking behaviour (*sensu* Barr and Babbitt 2002). Alternatively, observed differences are simply a result of a lower detection probability for *H. depressa* at low

abundances. Indeed, although the kick-sampling detected *H. depressa* at 100% of the sampling sites upstream of the waterfall, the video analysis only detected *H. depressa* at 44% of the sites where cameras were deployed. Moreover, whereas the kick-samples detected *H. depressa* at 38% of sites downstream of the waterfall, video analysis did not detect any *H. depressa* in this reach suggesting that kick-sampling has a higher *H. depressa* probability than does video analysis in the Krom River. Future studies should build on these preliminary observations by quantifying differences in detection probabilities of the different sampling methods for *H. depressa* and other amphibians in the CFE.

Because this was a comparative field study, and not a controlled experiment, the possibility of factors other than *O. mykiss* driving the observed pattern in *H. depressa* distribution cannot be ruled out. For example, it might be that *H. depressa* is naturally more abundant upstream of the Krom River waterfall, owing to a higher suitability of an unmeasured habitat parameter like flow velocity, temperature (which was only recorded at the time of sampling) and/or riparian habitat structure. Alternatively, predation pressure from potential predators not evaluated in this study, but present in the study area (JMS pers. obs. 2017), such as the river crab *Potamonautes*, or the common brown water snake *Lycodonomorphus rufulus*, might differ between the sections of river upstream and downstream of the waterfal and could also contribute to the observed differences in *H. depressa* abundance. Future studies should therefore assess a more comprehensive range of biotic and abiotic factors potentially influencing *H. depressa* abundance, and undertake complementary experiments to isolate the influence of *O. mykiss* predation.

In conclusion, this preliminary study provides correlative evidence that non-native *O. mykiss* might have a strong, negative impact on the *H. depressa* population in the Krom River, and highlights the role of waterfalls as barriers to non-native fish invasions and impacts in the CFE, as is the case elsewhere (Barr and Babbitt 2002; Karssing et al. 2012). The patterns reported here are consistent with the findings of Karssing et al. (2012), the only other published study of non-native fish impacts on amphibians in South Africa, yet it remains to be determined whether such patterns are repeated by other amphibian and non-native fish species in the CFE.
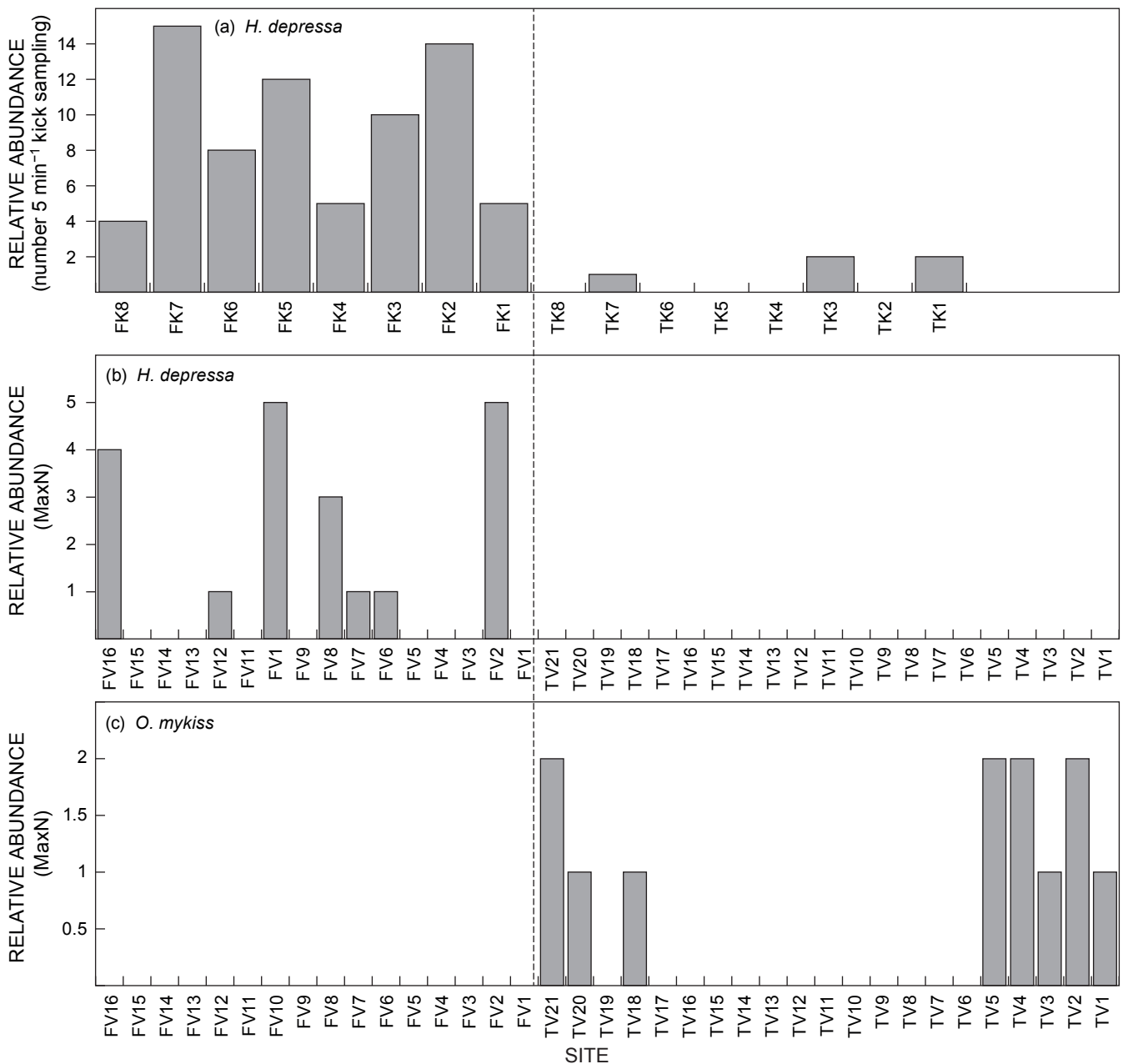
Figure 3: Relative abundance of *Heleophryne depressa* and *Oncorhynchus mykiss* at sites above and below the waterfall (dashed line) on the upper Krom River. Panel (a) shows relative abundance data based on kick-sampling (number of individuals recorded per 5 min sampling), and panels (b) and (c) show relative abundances of *H. depressa* and *O. mykiss* respectively based on video analysis (MaxN)

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