RESEARCH ARTICLE

In a rough spot: Declines in *Arthroleptella rugosa* calling densities are explained by invasive pine trees

Oliver Angus[1] | Andrew A. Turner[2,3] | John Measey[1]

[1]Department of Botany and Zoology, Centre for Invasion Biology, Stellenbosch University, Stellenbosch, South Africa

[2]CapeNature Biodiversity Capabilities Directorate, Cape Town, South Africa

[3]Department of Biodiversity and Conservation Biology, University of the Western Cape, Cape Town, South Africa

Correspondence
John Measey, Department of Botany and Zoology, Centre of Excellence for Invasion Biology, University of Stellenbosch, Natural Sciences Building, Private Bag X1, Matieland 7602, South Africa.
Email: john@measey.com

Abstract

Basing conservation interventions on evidence is important for justifying their associated cost and gauging their effectiveness. For amphibians, the number of studies available to support conservation action plans is limited. Here, we sought to determine the effect of invasive pine trees on the calling densities of a Critically Endangered frog species, endemic to the Western Cape province in South Africa. The Rough Moss frog, *Arthroleptella rugosa,* is an anuran restricted to a small patch of fire-driven fynbos habitat prone to invasion by Cluster Pines, *Pinus pinaster*. We use acoustic spatially explicit capture–recapture methods to estimate frog densities at multiple sites ($n = 12$) over ten years. Sites were classified as invaded or uninvaded by *P. pinaster*, and this information, along with the time since the last fire, were used as explanatory variables for frog density in a generalized linear mixed model. Frog densities were found to be significantly affected by *P. pinaster* invasion status. At invaded sites, there was a negative relationship between call densities and time since fire, while at uninvaded sites the same relationship was positive. These results confirm previous suggestions that invasive pine trees cause population declines in *A. rugosa*. Our findings can be used to support conservation interventions for *A. rugosa*, specifically the use of fire in an adaptive management context to control pine invasion across its range.

KEYWORDS
acoustic spatial capture recapture, adaptive management, amphibians, conservation evidence, fire, invasive plants, population declines

INTRODUCTION

Given the imperilled state of biodiversity across the globe, conservation interventions must greatly improve in efficacy if they seek to mitigate the threats posed to native species (Dirzo & Raven, 2003; Meredith et al., 2016). Many studies have highlighted the shift towards evidence-based conservation (see Sutherland et al., 2004), whereby actions are supported by the best available data. By using evidence to support conservation decisions, the effectiveness of these actions can be measured and used to inform future conservation interventions (Pullin & Knight, 2001). Often, management plans centre around certain groups of organisms or even a single charismatic species. Taxa peripheral to the focus of such conservation goals do not receive the same attention, despite facing threats of their own (Martín-López et al., 2009). Amphibians are one such taxon where the importance of evidence-based conservation is being recognized, given the threatened nature of the group globally (Hoffmann et al., 2010; Sutherland et al., 2004). In general, populations fluctuate naturally, but amphibian populations have seen far greater decreases in population sizes than overall increases (Alford & Richards, 1999). Thus, the use of evidence to support amphibian conservation is necessary given the reason for decline is often unknown or poorly understood (Cressey et al., 2015; Meredith et al., 2016).

Various causes for the global amphibian declines have been put forward, with habitat modification, infectious diseases and predation receiving the most support, while the effect of other factors like pollution and UV radiation are less understood (Vredenburg & Wake, 2007). Invasive species also pose a significant threat to amphibian populations, with 16% of all amphibian species considered to be threatened by

introduced alien species (Nunes et al., 2019). Invasive species were found to have been the greatest threat posed to extinct amphibians (Bellard et al., 2016). A meta-analysis by Nunes et al. (2019) found that invasive species significantly reduced fitness and performance of native amphibians, suggesting a strong negative effect on survivorship and reproductive success. Additionally, diversity and abundance declined in the presence of invasive species. There has been little success in mitigating the effects posed to amphibian populations by invasive species, stressing the need for studies aimed at elucidating the cause of declines so that evidence-based conservation measures can be implemented (Hoffmann et al., 2010; Meredith et al., 2016).

In contrast to alien animals, the interactions between non-native plants and amphibian populations are particularly poorly understood (Hoffmann et al., 2010; Nunes et al., 2019). However, a small number of studies have highlighted the potential for alien plants to degrade anuran breeding habitats through the displacement of native vegetation and their ability to alter natural hydrological processes (Le Maitre et al., 2002; Maerz et al., 2005) and as a driver of thermal effects (Garcia & Clusella-Trullas, 2019). Parris and Lindenmayer (2004) found that amphibian species richness declined significantly with the disappearence of native riparian vegetation, likely a result of overshading caused by non-native tree species. The reliance of most amphibians on water, for at least one of their life stages, makes amphibians particularly vulnerable to changes in normal hydrological conditions (Nunes et al., 2019). Another consequence of alien plant invasion is the synergy it has with other drivers of extinction (Sodhi et al., 2008). One such interaction involves that of fire and invasive alien plants, where the latter can alter the intensity and frequency of fire regimes through increased fuel loads (van Wilgen et al., 2010; van Wilgen & Richardson, 1985). In general, there is a lack of understanding surrounding the effects of both fire and invasive plants on local amphibian populations, where several studies have reported responses in opposite directions (Kirkland et al., 1996; Measey et al., 2021; Penman et al., 2006; Pilliod et al., 2003).

Fynbos is a biodiverse, evergreen, Mediterranean, shrubland vegetation type located at the south-western most region of Africa (Mucina & Rutherford, 2006) and is particularly threatened by the invasion of woody alien plants (Richardson et al., 1992). In fynbos, the encroachment of alien trees into amphibian breeding habitats is thought to threaten their survival, although this impact has never been quantified (Brooks et al., 2004; Le Maitre et al., 2002; Measey et al., 2011; Turner & De Villiers, 2017). In conjunction with increased water uptake and general habitat degradation caused by these trees, there is also the threat posed by the synergism between tree invasion and fire. Invasive trees cause an increased fuel loading in fynbos habitats, making fires burn with greater intensity and frequency (Brooks et al., 2004). Fire is thought to considerably affect the population dynamics of native amphibians in the region (Measey et al., 2011, 2019), and long-term monitoring can measure the response of amphibian populations. Quantifying the impact that invasive trees have on native amphibian populations can allow evidence-based conservation initiatives to be implemented. These initiatives include large-scale tree clearing or managed fires.

A novel approach to monitor calling frog populations uses call density from microphone arrays and analysis via acoustic spatially explicit capture–recapture (aSCR) to accurately determine calling animal density (Stevenson et al., 2015). The Rough Moss frog, *Arthroleptella rugosa*, is an ideal candidate for acoustic monitoring using call densities as they are extremely small and completely concealed in seeps within dense fynbos vegetation making them very difficult to observe. Call densities can

be assessed in relation to an ongoing pine invasion of the fynbos habitat of this micro-endemic on the Klein Swartberg. If *A. rugosa* call densities are found to decline following the invasion of a seepage, this can be used as evidence to provide impetus for the continuation of alien clearing. The aim of this study is to investigate the effect of invasive alien pine trees on *A. rugosa* call densities. To do this, we created a generalized linear mixed model to determine whether time since fire (TSF), invasion status and season explain *A. rugosa* call densities as measured by aSCR surveys. We expect that calling density in *A. rugosa* will be significantly affected by the synergism between invasion status and TSF, and therefore an interaction term is included. Specifically, pine-invaded calling sites will see a declines in call densities with more time since the last fire, while calling densities at uninvaded sites should respond in the opposite way.

MATERIALS AND METHODS

Study species

The genus *Arthroleptella* are small frogs in the family Pyxicephalidae endemic to southwestern South Africa. They are strictly montane, associated with dense vegetation growing in and around seepages in fynbos (Turner & Channing, 2008). The Rough Moss frog, *Arthroleptella rugosa* Turner & Channing, 2008 has been monitored post-fire in order to gauge its recovery (Turner & De Villiers, 2017). Measey et al. (2011) ranked *A. rugosa* second with respect to research priority for conservation importance of South Africa's frogs. Monitoring of *A. rugosa* populations is important, given its status as a micro-endemic on the Klein Swartberg Mountain, with an estimated Area of Occupancy (AOO) of 0.9 km^2 and an Extent of Occurrence (EOO) of 2.3 km^2 (IUCN SSC Amphibian Specialist Group and South African Frog Re-Assessment Group, 2016). This species is confined to the Klein Swartberg Mountain in Caledon, which is itself an island of montane fynbos surrounded by farmland which was once renosterveld—another vegetation type in the Cape Floral Kingdom (Mucina & Rutherford, 2006; Turner & Channing, 2008). Notably, this is the only species in the genus occurring on the Klein Swartberg Mountain and has a unique call which allows reliable acoustic identification (Turner & Channing, 2008). Aspects of *A. rugosa*'s life history also make it particularly vulnerable to plant invasions. Firstly, members of the genus *Arthroleptella* have relatively low dispersal capabilities when compared to other anurans (Rebelo & Measey, 2019; Turner, 2010). They are thought to be highly philopatric, meaning they could be considered more vulnerable to disturbance (Turner, 2010). Secondly, larval stages of *Arthroleptella* undergo direct development (De Villiers, 1929), making them vulnerable to drier conditions induced by invasive alien trees (Le Maitre et al., 2002; Turner, 2010). Given the poor mobility of the species, this makes dispersal to other mountains effectively impossible (Rebelo & Measey, 2019; Turner & Channing, 2008). Much of the Rough Moss frogs' habitat is invaded by Cluster Pine, *Pinus pinaster*, which degrades the seepages where it occurs by reducing soil moisture content (Gaertner et al., 2014; Turner & Channing, 2008). The threat posed by these alien pines includes increased fire intensities and shortened fire return rates. Together with its small AOO and EOO, these threats have given *A. rugosa* the IUCN conservation status of Critically Endangered, with a high risk of extinction (IUCN-ASG and SA-FRoG, 2016). It is important that these perceived threats be measured, so that management plans and stakeholder agreements can be established based on evidence.

Study area

The study area is located on private farms across the Klein Swartberg (see Figure 1) near Caledon (34°12′S 19°32′ E), meaning that *A. rugosa* does not occur on any formally protected area. The dominant vegetation type is Overberg Sandstone Fynbos, which is characterized by a dense proteoid layer reaching no higher than 1.5 m (Mucina & Rutherford, 2006). Roughly one sixth of the mountain has been invaded by *Pinus pinaster* and *Hakea sericea*, although invasion by the former species is more intense and can reach heights of over 20 m. Rough Moss frog habitat patches, which are associated with seepages or moist areas, are unevenly distributed across the mountain ridge (Stark et al., 2022). Until 2019, populations were only known from the southern slopes of the mountain (Stark et al., 2022), however, in 2020 another population was discovered on the northern slopes of the Klein Swartberg (A.A. Turner, K. Lynch & O. Cowan pers. obs.).

Call density estimates

Acoustic spatially explicit capture–recapture (aSCR) is a monitoring technique used to accurately determine animal densities and even population sizes for a variety of species that call (Stevenson et al., 2015). The method uses capture histories to generate a detection function, which describes the decreasing probability of detecting a call as the horizontal distance between the microphone and the call source increases (Measey et al., 2017; Stevenson et al., 2015, 2021). The detection function allows for the calculation of density of calls detected by the microphone array at any point within the effective survey area (ESA; Measey et al., 2017). There are several advantages of using aSCR over other acoustic methods. Firstly, it does not require a skilled operator as the setup of the microphones is relatively straightforward. Secondly, large quantities of acoustic data can be generated within a relatively short period of time, improving the power of the statistical analysis. Thirdly, aSCR incorporates additional information, like time-of-arrival information (ToA), which greatly improves the ability to infer locations of emitted calls, thus improving the accuracy of density estimates (Stevenson et al., 2015). Like other acoustic methods, using aSCR to collect data means that there is no handling, catching or physical tagging of
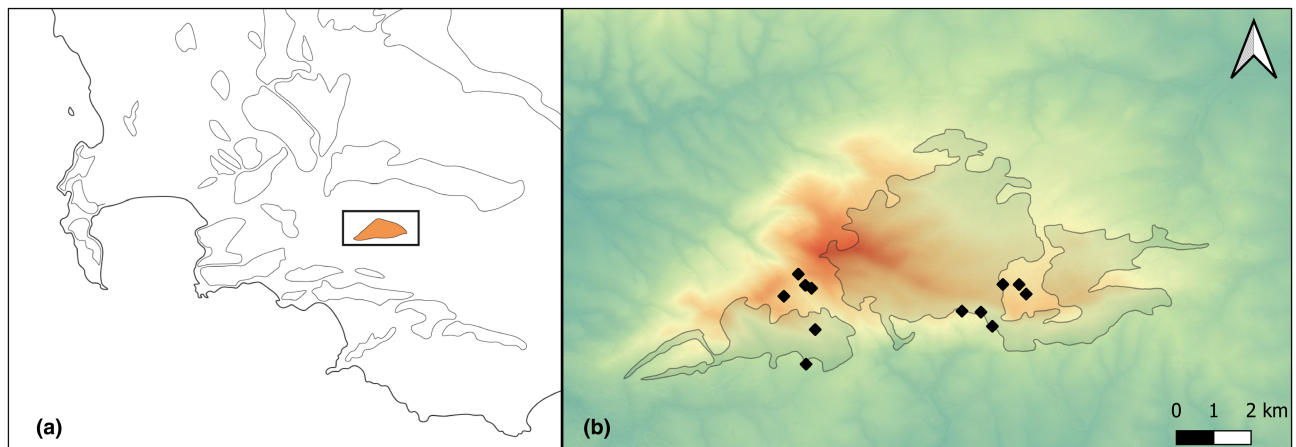

FIGURE 1   (a) Mountain ranges of the southwestern cape, South Africa. The dashed box indicates the boundary box of the graphic on the right. (b) The Klein Swartberg Mountain, an isolated inselberg located near Caledon, and the home range of *Arthroleptella rugosa*. Shaded area shows the extent of the invasion of *Pinus pinaster*, while numbered circles represent microphone arrays. Source: OpenStreetMaps.

individual animals on which traditional capture–recapture techniques rely. However, it also comes with caveats as we assume that all males in the population are calling so that call density represents true male density and that male and female ratios are equal (Measey et al., 2017).

In this study, surveys were made exclusively over the winter months from May to October (from 2012 to 2021) when male Rough Moss frogs are heard calling. Depending on the number of frogs thought to be heard calling, two different methods of acoustic surveying were used:

Aural surveys

Louw (2018) found the accuracy of aural estimates to be substantially greater than those generated from aSCR analysis when the number of frogs calling was less than six. Thus, if an observer determined there to be five or less frogs calling, a microphone array was not deployed and instead calling individuals were counted by listening to calls (Louw, 2018). Aside from improvements to accuracy, alternating between the two methods saves researchers a considerable amount of time in the field thereby increasing sampling coverage on a single day of surveying. Because members of the genus *Arthroleptella* are reliable callers, listeners can estimate small populations in 10–20 min. To calculate male densities, the number of estimated individuals were divided by the seepage area in which males were calling. Seepage bounds used were the same as those used in the study by Stark et al. (2022), and newly made for additional sites. All seepage bounds, irrespective of method, were determined by creating a GPS track by walking around the seepage area in an anti-clockwise fashion, making sure to always hear frogs calling on the left. The completed track was saved and later converted into a shapefile so that its area could be calculated.

aSCR surveys

If the number of frogs calling at a particular site was determined to be greater than five, a microphone array was set up to record for 30–40 min. Equipment used in a single acoustic survey included one TASCAM DR-680 MKII Portable Field Audio Recorder (Tascam; TEAC), six omni-directional AT8004 microphones (Audio-Technica), six XLR cables and six 1 m dowel sticks with fitted microphone holders (see Stark et al., 2022). The placement of each microphone in the array was such that there was a minimum distance of 4 m from the recorder and 2 to 5 m between the adjacent microphones. The placement of microphones was done in a way to avoid the array taking a regular circular shape, which circumvents the potential for calls to be equidistant from all microphones (see Stevenson et al., 2015). Recordings were made for 40 min, with the first 10 min of each recording being discarded to prevent impacts on calling from setting up the acoustic array. After termination of the recording, the GPS coordinate of each microphone was recorded using the waypoint averaging feature on a Garmin eTrex® 20X. The distance between every microphone pair was measured to the nearest centimetre. The GPS Cartesian coordinates are used as priors together with the linear measures in the microphone location estimation (see below), to accurately determine the relative position of each microphone in the array (Stevenson et al., 2015). Array positions are used when calculating the Normalized Difference Vegetation Index (NDVI) mean for each site (see below). The polygons were kept the same for the sites surveyed in Stark et al. (2022) study. Recordings were made on calm days without rain and/or severe winds.

aSCR analysis

Four inputs were required to fit an aSCR model, namely a file containing call detections, the Cartesian coordinates of each microphone in the array used to make that recording, the mean call rate of the study species, and the time range of the recording subsample. The packages ascr (Stevenson et al., 2015), parallel (Rossini et al., 2007), secr (Efford, 2011) and data. table (Dowle et al., 2019) were used for the final analysis within R (R Core Team, 2021). The methodology to obtain each of the measures described in this section are described individually in the below sections (a) to (d).

a. Call detections

The application, Raven Pro v.161 (K. Lisa Yang Center for Conservation Bioacoustics, 2019) was used to detect *A. rugosa* calls in each 6-channel audio recording. This was done using the Band Limited Energy Detector feature, which identifies structures in the spectrogram that meet specific parameters relating to frequency bands and call duration. These parameters need to be specified by the user and require some ground truthing as environmental sounds can be falsely classified as frog calls. We manually determined these parameters by starting with an arbitrary set of values and adjusting them so that the number of false-positive detections were reduced to a point where additional refinement has little to no effect on the number of false-positive detections. A 40 min recording of an *A. rugosa* chorus was used to conduct these preliminary tests. Peak Amplitude and Begin File Sample were included as measurements for each detection. Preliminary testing in Raven found the following parameters to best predict *A. rugosa* calls; a minimum frequency of 3000 Hz, a maximum frequency of 4500 Hz, a minimum duration of 0.02133 s, a maximum duration of 0.11733 s and a minimum separation of 0.02133 s.

b. Microphone location estimation

The GPS coordinates of each microphone in the array are converted to cartesian coordinates and corrected according to the straight-line distances between each microphone. These new coordinates, as well as the microphone distances, are used to estimate each microphone's location within the array.

c. Call rate

The aSCR model requires a vector of call rates collected independently from the main acoustic survey, measured in calls per unit time. The *A. rugosa* call rates (calls.min^{-1}) used in this study had been determined by Stark et al. (2022), with a mean of 21.86667 calls.min^{-1} (σ^2 = 235.2667).

d. Subsample

To create a capture history object, a 10-min subsample from each recording was used. The position of each subsample in the recording was manually selected to reduce the amount of environmental noise, as this has potential to create false-positive detections. The first 10 min of each recording were avoided, as calling behaviour was presumed to have been affected by the presence of the researchers in the seepage area. We assume that call density represents the density of males in each population and that the population has equal numbers of calling males and non-calling females.

Model fitting

All aSCR analyses were done in R (R Core Team, 2021) using the package 'ascr' developed by Stevenson et al. (2021). The fit.ascr() function generates an estimate for the density of calls (D) within the ESA using a maximum-likelihood approach. From D, the density of animals (Da) can be derived. The ascr model used ToA information, with an accuracy of 2.083×10^{-5} s, to better estimate call locations. When a call is detected on more than one microphone, there are differences in the ToA of that call across the microphone array as ToA is dependent both on the speed of sound in air ($330\,m.s^{-1}$) and distance between the microphone and the call source. This information is then used, together with the actual locations of the microphones, to better determine the location of the frogs in the ESA. Parametric bootstraps were used to estimate the standard errors of D and Da at each survey area and were run for 300 iterations. The Monte Carlo errors from the bootstrap were kept under 0.05.

In R, a preliminary analysis revealed high overdispersal of density estimates. The package bestNormalise (Peterson, 2021) suggested a Yeo Johnson transformation to normalize the data. Upon visual inspection of the data, and through a Shapiro–Wilk test, the transformed data were found to be non-parametric. As a result, a generalized mixed model with a negative binomial data family was chosen. The package glmmTMB (Brooks et al., 2017) was used to create the model. The model negative binomial 2 was selected over the model negative binomial 1 using the methodology of Bolker et al. (2012). The model with the lowest AIC was chosen using dredge in the package MuMIN (Barton, 2009).

Invasion status

The mean NDVI inside the seepage boundaries at each site on the Klein Swartberg was used to determine the degree of pine invasion. A cut-off was used to distinguish native and non-native vegetation, namely fynbos and pine. This method has been used with high accuracy in prior studies to stratify vegetation into classes corresponding their greenness (Holden et al., 2021; Jafari et al., 2017; Kumar et al., 2007). NDVI is measure of vegetation greenness or vigour, incorporating information from two different bands, visible and near-infrared reflectance (Weier & Herring, 2000). Due to the sclerophyllous nature of fynbos, dark green pines are expected to have significantly higher NDVI estimates as they reflect significantly more near-infrared radiation than visible radiation (Rebelo et al., 2021).

Google Earth Engine (GEE) was used to extract mean NDVI values for each site. GEE is a development environment which allows a user to extract data from satellite imagery using a JavaScript API. An NDVI data set derived from Landsat 7 satellite imagery was used courtesy of the US Geological Survey, where pixels represented an area of $900\,m^2$. A cut-off NDVI value was used to separate vegetation into two classes: pine (invaded) and fynbos (uninvaded). To do this, satellite imagery and photographs taken in the field were compared with pixel values from the NDVI data set to ground truth the chosen threshold. Mean NDVI values for each site were calculated using the Maximum Value Composite (MVC) technique, which improves the accuracy of NDVI estimates. The MVC procedure creates an image where only the maximum pixel values from a NDVI time series are retained, and the resulting MVC image is composed of each pixel's maximum value. MVC requires multi-temporal satellite data. An NDVI data set derived from LANDSAT satellite imagery was used, where images were taken 8 days apart from the 1st January to the 30th April for each year. This time range

corresponds to the summer months, where the growth experienced by fynbos is at its lowest resulting in an increased difference in greenness between the alien pines and native fynbos.

Time since fire (TSF)

In GEE, a MODIS-derived burnt area data set, MCD64A1.006 by Giglio et al. (2015), was used to extract the date of the last fire at each site. The number of days between the last fire at the date of each recording were then calculated.

Season

Season was recorded as the number of days since the beginning of the year in which the recording was made and the sampling date. Season was included as a fixed effect in the generalized linear mixed model. Calling activity in moss frogs is known to vary with season (Measey et al., 2017).

Analysis

A generalized linear mixed model (GLMM) was used to determine the effect of the time since fire (TSF), invasion status and season on calling frog densities, with the variable site being included as a random effect:

Call density $\sim$ TSF x Invasion status + Season + (1 | Site)

Full R code and data are supplied are available online (Angus et al., 2022).

RESULTS

Call density estimates

Between 2012 and 2021, a total of 56 acoustic surveys were conducted across 13 sites on the Klein Swartberg. Of these, 35 followed the aSCR methodology, and the remainder utilized the aural technique. The number of replicates ranged from one to three across each year of sampling. A mean male density of 410 individuals per hectare ($\sigma^2 = 252\,300$) across all 56 observations was observed.

Invasion status

Through the comparison of pine invasion in LANDSAT satellite imagery and pine cover as predicted by NDVI, a cut-off of 0.571 was used. Pixel values equal to or higher than 0.571 represent invaded areas of $900\,m^2$. Across all 56 acoustic surveys, half were at invaded sites and half at uninvaded sites.

Time since fire

The burn-area MODIS-derived data set was used to determine the last fire which occurred at each size. The fire on 22nd of January 2012 was found to be the last burn date for every site. Thus, the time since fire was the number of days between the date of the recording and 22nd of January 2012.

Generalized linear mixed model

When ranked by AIC, the best model included only the interaction between invasion status and time since fire (TSF; Table 1). A Wald chi-square test found the effect of invasion status to be statistically significant ($\chi^2 = 16.583$; $p < 0.001$), as well as the interaction with TSF ($\chi^2 = 17.674$; $p < 0.001$). At sites invaded with Cluster Pine, the relationship between frog density and TSF was negative, but the same relationship was positive for sites uninvaded by Cluster Pine. Both invasion states, and their interaction with TSF, were statistically significant (Figure 2).

DISCUSSION

In pine-invaded areas, frog call density strongly decreased over time, and in uninvaded sites density increased over time since the last fire (TSF). These results show that once a seepage becomes invaded, the density of frogs begins to decline with time. While in uninvaded sites, frog calls increase in density following a fire. It follows that fires reduce moss frog calling densities, in the short term (which has been observed elsewhere J. Measey and A.A.Turner pers. obs.). But because the 2012 fire impacted all sites monitored this could not be tested with our data set. Evidence for the impacts of alien plants on amphibian populations is equivocal, with the last meta-analysis showing a positive mean effect size for amphibian fitness and performance in the presence of alien plants (Nunes et al., 2019). Similarly, the impacts of fire on amphibian populations have been reported as having both positive and negative effects (Kirkland et al., 1996; Measey et al., 2021; Penman et al., 2006; Pilliod et al., 2003). This failure to find simple effects could be due, in part, from a lack of investigations that consider the synergy of additional stressors. Especially when amphibians co-evolve within fire-driven habitats, we should expect a level of stochastic fluctuation in populations that are ultimately able to recover between fire cycles. However, invasive plants or other additional stressors may result in fire crashing populations through synergistic effects, as we found in our study site.

Our data confirm previous anecdotal observations that the invasion of pine trees on the Klein Swartberg is causing a serious decline in the populations of *A. rugosa* and that this is the opposite to population trends at uninvaded sites. Evidence for *A. rugosa* decline can be used to support future conservation interventions for this species, as well as other moss frogs in fire-driven fynbos habitat that appear to be threatened by the encroachment of invasive trees. The results from this study are important

TABLE 1 Results of generalized linear mixed models, ranked by Akaike information criterion (ΔAIC), on the effect of the time since fire (TSF), invasion status of cluster pines and season on calling densities of *Arthroleptella rugosa*, with the variable site being included as a random effect

Model	ΔAIC	df	Weight
Time since fire x Invasion status	0	6	0.761
Time since fire x Invasion status + Season	2.62	7	0.205
Invasion status	7.56	4	0.017
Time since fire + Invasion	9.73	5	0.006
Season + Invasion status	9.79	5	0.006
Season + Invasion status + Time since fire	12.04	6	0.002

The best model contains only the interaction between TSF and invasion status. Degrees of freedom (df) and the model weight (weight) are also shown.
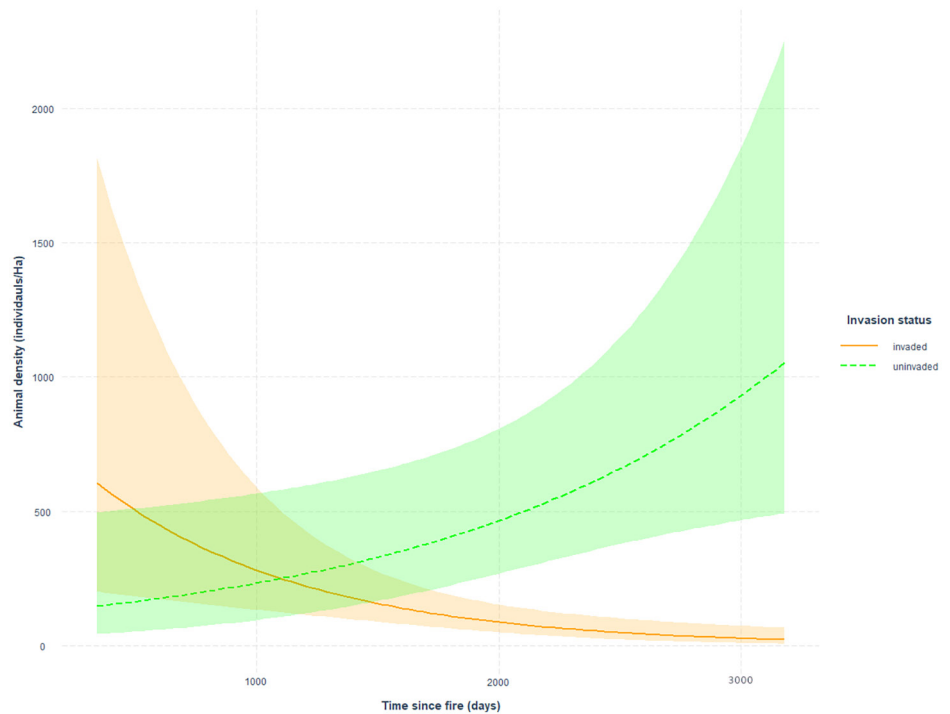
FIGURE 2 Interaction effect between the density of calling rough Moss frogs (*Arthroleptella rugosa*) and time since fire. At uninvaded (green) call density declines with increased time since fire, whereas at sites invaded with *Pinus pinaster* (orange) a density decrease is observed. The shaded colours depict 95% confidence intervals.

given the lack studies quantifying the effect of perceived threats on amphibians (Angulo et al., 2011; Christie et al., 2020). Our results also show that *A. rugosa* populations can recover after a fire if their habitat remains uninvaded. This evidence is important in that it explains the persistence of moss frogs in fire-prone fynbos vegetation and supports the use of fire as a conservation tool in controlling the invasion of pines in seepages occupied by *A. rugosa*.

The invasion of pines in seepage habitat has had a significant effect on *A. rugosa* populations on the southern slopes of the Klein Swartberg where the pine invasion is the most intense. There are three principal reasons as to why pines would cause declines in *A. rugosa*. Firstly, invasive trees are known to significantly decrease soil moisture content through increased transpiration rates, and this effect is even greater in riparian zones (Dzikiti et al., 2013; Richardson et al., 2007). Consequently, amphibians are particularly vulnerable given their reliance on suitable microenvironments to regulate their water balance (Carvalho et al., 2010; Wells, 2010). Secondly, pine trees alter stream and seepage morphology via bank erosion and channel widening, thus reducing the seepage width and increasing water flow (Esau, 2005). Habitat degradation by invasive pines is likely to threaten populations of *A. rugosa* through the removal of suitable microhabitats, since the species depends on seepages for survival and reproduction. Thirdly, temperature of microhabitats occupied by moss frogs is likely to decrease in the shade of pine trees, and reduced temperatures could increase development times for their directly developing eggs (see Garcia & Clusella-Trullas, 2019). Our observations of the invaded sites on the Klein Swartberg suggest that transpiration and channel narrowing are seriously decreasing the width of seepages.

Aside from the direct threats, pines also threaten *A. rugosa* populations indirectly via the interaction they have with fire. An increase in

biomass of pine trees results in fires of greater intensity (van Wilgen & Richardson, 2012). This may have consequences for recovery of populations post-fire, and if densities are already low because of the original invasion, then the effect of more intense fires may even be greater. Frogs of the genus *Arthroleptella* are known to avoid fire by sheltering in shallow burrows (Measey et al., 2021). Increasing intensity of fires, in the presence of woody invasive plants, may raise soil temperatures above the level at which frog refugia can protect individuals. While this study could not test this, the seepages on the southern slopes were already invaded before the 2012 fire (A.A. Turner & J. Measey pers. obs. but also through inspection of NDVI values pre-2012) and those populations have persisted (this study). However, in the post-fire period, densities of those populations on the southern slopes never reached the densities observed at the uninvaded sites in the other two catchments. This might suggest that those populations on the southern slopes started off small and remained small because of the intensity of the 2012 fire and the pine invasion post-fire, although we cannot discriminate between these potential causes. Because populations of *A. rugosa* were found to be relatively small, there is expected to be increased susceptibility to declines caused by stochastic processes, like droughts or wildfires (Blaustein et al., 1994; Murray et al., 2017).

Given the small range of the Rough Moss frog, it is concerning that we found one site where a local extinction has already occurred. Both upstream and downstream sites may function to help recolonize this area after controlled fires remove the pines. A molecular study on another small direct developing frog suggests an ability to move within watersheds (Measey et al., 2007). Dispersal abilities of *A. rugosa* can be assumed to be poor if they are like other members of the genus (see Rebelo & Measey, 2019). Despite the small distances between within-catchment populations, reduced vagility means that even if habitat is continuous, movement between seepages is probably low. Moss frogs from the northern slopes of the Klein Swartberg are therefore less likely to colonize slopes on the south, compared with those at adjacent sites on the southern slopes. Pines are most easily controlled by fires with frequencies that prevent successful reproduction (<9 years, A.A. Turner pers. obs.), but Rough Moss Frog populations also need time to recover (i.e. approximately every 7 to 8 years, see Figure 2) until such time as the pine invasion is extirpated. Thereafter the burn frequency can be allowed to revert to average fire return intervals of 12–15 years (van Wilgen et al., 2010). We suggest extra safeguards for these threatened populations using block burning with fire breaks separating different subpopulations, and manual removal of pines around breeding sites to reduce fire intensity. This adaptive management response monitors fire return interval against the management goal of maintaining threatened species populations.

In conclusion, this study provides evidence for local population declines in *A. rugosa* associated with invasion of pine trees into their seepage area habitats. Moreover, we find that there is an interaction effect between the invasive pines and fires that synergistically deplete moss frog populations. These results can be used to support conservation interventions, both for this species and others in fire-driven habitats facing similar threats from invasive trees. Although conservation action in the form of controlled burns will negatively impact populations of *A. rugosa* in the short term, carrying out block burns on different burn cycles will ensure control of the invasive pine trees and mitigate this threat in the long term. Despite caveats associated with the calculations to estimate moss frog density using aSCR (see Measey et al., 2017), this method objectively measures call density, while archived recordings remain available for re-analysis in the future. Hence, data from this study will act

as an important baseline to measure the efficacy of future conservation actions, in particular the use of controlled burns to reduce pine invasion in *A. rugosa* habitat.

AUTHOR CONTRIBUTIONS

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

DATA AVAILABILITY STATEMENT

All data and code are openly available at https://doi.org/10.17605/OSF.IO/A94CD

ORCID

Oliver Angus https://orcid.org/0000-0002-9791-9480
Andrew A. Turner https://orcid.org/0000-0003-1004-5723
John Measey https://orcid.org/0000-0001-9939-7615

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