Spawning and non-breeding activity of adult giant bullfrogs

(Pyxicephalus adspersus)

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Abstract.—Populations of the Near-Threatened giant bullfrog (*Pyxicephalus adspersus*) have been poorly monitored due to the unpredictable appearance of this species aboveground. To better understand the activity of *P. adspersus* we quantified spawning by a population during five summers, and the activity of twenty adult frogs radio-tracked at the same site ca. twice weekly during the first three summers. In addition we examined animal activity, and population spawning in relation to meteorological variables, day of season, and moon light. During the six-month summer period, males and females, respectively, spent 10 ± 7 and 3 ± 2 days at water, and 22 ± 5 and 13 ± 5 nights active. Greater proportions of radio-tracked animals moved overland, and/or foraged at night, around full moon, after heavy rainfall, when cooler, and less windy conditions prevailed. More animals were found at water, or on land during the day, and population spawning was more likely, earlier in summer, following heavier rainfall. Spawning occurred most frequently, in descending order, during December, January, and November, and was triggered by 40 ± 16 mm rain in 24 h. Spawning events lasted 2 ± 1 days, but were prolonged around full moon. Numbers of males at spawning events varied between 30 and 500 males, and were positively correlated with the previous day's rainfall. Annually 6 ± 1 spawning events occurred, and numbers of annual spawning events were positively correlated with total summer rainfall. Results of this study show that single counts of spawning adults will often result in gross underestimates of population size, and therefore, long-term adult counts are necessary to detect real population trends. Due to the unpredictable activity of adults, however, it may be more practical to monitor, within certain areas, the number of aquatic sites where breeding occurs, for improved conservation management of *P. adspersus*.

Although photoperiod and the lunar cycle provide predictable cues that amphibians may use to synchronize their behaviour (Fitzgerald & Bider 1974; Both *et al.* 2008; Canavero & Arim 2009; Grant *et al.* 2009), the activity of many species is ultimately dictated by unpredictable climatic variables because of their permeable skin and ectothermic bodies (Duellman & Trueb 1994). Among these, precipitation tends to exert the greatest influence (Bulger *et al.* 2003; Lemckert & Brassil 2003), but temperature (Howard 1978; Van Gelder *et al.* 1986), humidity (Bellis 1965; Bartelt *et al.* 2004), wind (Penman *et al.* 2006; Philips *et al.* 2007), light intensity/cloud cover (Jameson 1955; Blankenhorn 1972), and barometric pressure (Blankenhorn 1972) can also significantly affect amphibian activity. Amphibians in dry and/or cold environments are most limited by suitable warm, wet conditions, making it difficult to predict their behaviour or even their presence (e.g., Bulger *et al.* 2003; Bartelt *et al.* 2004; Goldberg & Schwalbe 2004). This can represent a serious challenge for effective conservation management of threatened amphibian taxa (e.g., Penman *et al.* 2006).

Giant bullfrogs (*Pyxicephalus adspersus*) are large frogs (males: ~ 400-1 000 g; females: ~ 90-300 g; Cook 1996) inhabiting arid to subtropical grassland and savannah in southern Africa (Channing 2001). Individuals remain underground in a state of torpor except in summer when triggered by heavy rain to spawn explosively in shallow, ephemeral water bodies (Balinsky & Balinsky 1954; Grobler 1972). Little is known about the non-breeding biology of this species apart from its diet, and burrowing physiology (Loveridge & Withers 1981; Van Aardt 1992; Du Preez & Cook 2004; Secor 2005). Currently, *P. adspersus* is listed as Near-Threatened in South Africa (Minter *et al.* 2004; IUCN 2008), where habitat

loss and other factors have led to local population declines of 50-80%, particularly in Gauteng Province (Harrison *et al.* 2001). Unfortunately, due to the highly unpredictable activity of *P. adspersus*, the species has been subject to limited field-based research and monitoring (Jacobsen 1989; Kok *et al.* 1989; Van Wyk *et al.* 1992; Cook 1996). This has hindered accurate assessment of the size, and breeding success, of important historical populations (e.g., at Glen Austin and Bullfrog pans near Johannesburg). The resulting lack of demographic information for the species has impeded assessment of its conservation status, and provides a weak baseline for evaluation of efforts to protect threatened populations.

In the present study we aimed to examine the spawning and non-breeding activity of adult *P. adspersus* in relation to meteorological variables, to contribute towards improved detection, monitoring, and conservation management of this species. Specific objectives of the study were to:

- 1) Quantify the annual spawning, and non-breeding activity of adult *P. adspersus*.
- 2) Determine whether males and females exhibit different patterns of diurnal or nocturnal activity.
- 3) Examine the diurnal, nocturnal, and spawning activity of adult *P. adspersus* in relation to meteorological variables, day of season, and moon light.
- 4) Infer consequences of the results on the conservation management of *P. adspersus*.

MATERIALS AND METHODS

Study site.—The study site was located in Diepsloot, Gauteng Province, South Africa (2528CC: 25°56'23.59"S, 28°01'21.88"E; 1 427-72 m a.s.l.), and comprised ~ 100 ha of degraded Egoli Granite Grassland (Mucina *et al.* 2005). Local bullfrogs bred in three small (~ 0.2-0.9 ha), shallow (< 2 m deep), human-created, seasonal dams (named Dams 1, 2 and 3), situated in the southern section of the site. The site is representative of many areas where *P. adspersus* faces encroaching urbanization on remaining fragments of undeveloped habitat. We collected data on *P. adspersus* at the site between 1 October and 31 March in the seasons 2003/2004, 2004/2005, 2005/2006, 2006/2007, and 2007/2008 - hereafter referred to as Summers 1-5.

Radio-tracking.—In Summers 1-3, we haphazardly selected 10 males and 20 females for radio-tracking from adult *P. adspersus* caught for a separate mark-recapture study. We captured frogs by hand or with a hand-held net during spawning events in Dams 1 and 2 (*P. adspersus* did not favour Dam 3). Animals were each implanted off-site with a radio-transmitter by a veterinary surgeon, monitored overnight, and released the next day at their capture sites. The transmitters and surgical protocol are described in Yetman & Ferguson (in press).

Tracking was conducted between 06h00 and 18h00 on average twice per week, with additional surveys undertaken in response to rainfall events. A four-element Yagi antenna and AVM LA12 receiver (AVM Instruments, Champagne, Illinois) were used to detect radio-tagged frogs. Between three and 16 animals were tracked on a day. When a frog was

located, we recorded its geographic coordinates (with a global positioning system, accurate to 4 m), and the date, time, and behaviour observations. An animal's behaviour was classified as: buried (inside an open or closed burrow); present at a spawning event (regardless of whether an animal mated); guarding offspring (resting < 1 m from or aggressively defending eggs or tadpoles); resting at water (when an animal was found in or < 1 m from a dam or other inundated depression, and spawning or parental care of offspring was not observed); or resting on land (resting aboveground > 1 m from water, usually under vegetation). Data on the diurnal activity of radio-tracked animals were obtained for 129 days during Summers 1, 2, and 3.

Evidence of nocturnal frog activity.—No nocturnal radio-tracking was performed because the area was considered unsafe at night. However, in Summer 3 we deduced the nocturnal emergence of animals from their burrows when the radio-tracked location of individuals changed overnight, or when a small stone placed on the plug sealing an animal's burrow had disappeared or been displaced off the plug between consecutive days of tracking. Pearson (1955) used chalk dust for the same purpose, but in our study stones were used as less conspicuous markers to prevent animals from being harvested for human consumption (Du Preez & Cook 2004). Data on the nocturnal activity of radio-tracked animals were obtained for 31 nights in Summer 3.

Monitoring of spawning.—All aspects of breeding by *P. adspersus* take place at the edge of ephemeral wetlands where the water is most shallow and warm, and thus favourable for tadpole development (Cook 1996). We therefore inspected the edge of the seasonal dams

on foot, usually every day that animals with radio-transmitters were tracked in Summers 1-3, and after rainfall events of 20 mm or more rain at the study site in Summers 4-5.

Evidence of spawning by *P. adspersus* included observation of calling, fighting and/or mating adults, eggs, tadpoles, and/or newly-metamorphosed froglets – which were clearly visible at the water's edge and are highly diagnostic (Du Preez & Carruthers 2009).

When a spawning event was observed, males at Dams 1-3 were counted in groups of approximately 10 individuals, if possible at different times of the day for each day that adults were seen calling, fighting and/or mating. Thus the largest count of males, and the duration of each observed spawning event was determined. Females were not counted because their smaller size and inconspicuous behaviour (Channing *et al.* 1994; Cook 1996) made them difficult to detect.

When eggs, tadpoles or newly-metamorphosed froglets were found without spawning having been observed, we estimated the date of spawning from the development of offspring (Gosner 1960; Van Wyk *et al.* 1992; Haas 1999) and measurements of daily rainfall from Diepsloot Nature Reserve. Hence, for Summers 2-5, we estimated the annual number of spawning events as the sum of all spawning events that were observed or revealed by eggs, tadpoles or froglets. The total number of spawning events in Summer 1 could not be determined since the study site was found in late mid-summer (January 2004).

Meteorological data.—For periods when *P. adspersus* spawned at the study site, we obtained measurements of daily rainfall from a local resident who lived < 100 m from the

dams. These data were supplemented with rainfall measurements taken at 07h00 daily in gauges at five different localities in Diepsloot Nature Reserve, which lies ~ 6 km west of the study site (25°55'36.35"S, 27°57'56.09"E). We treated 1 October as the first day of season (DS), and used a lunar calendar (South African Astronomical Observatory) to determine the number of days since full moon on specific dates. Days since full moon were assigned to one of eight lunar phases, ranked from 0-4 depending on the amount of moon light (ML; Table 1, adapted from Grant *et al.* 2009).

We obtained daily measurements of maximum and minimum atmospheric temperature (MaxT and MinT in °C), relative humidity (RH in %), wind speed (WS in m s⁻¹), and atmospheric pressure (P in hPa) from a weather station in Irene, situated ~ 19 km east of the study site (25°54'36.00"S, 28°12'36.00"E). Missing temperature data in November and December 2005, and October 2006 were substituted with daily MaxT and MinT measurements from a weather station in Kempton Park (situated ~ 31 km south-east of the study site; 26°08'60.00"S, 28°13'48.00"E). These measurements were corrected by 0.4-3.0 °C depending on the mean monthly difference in each variable between the two weather stations during Summers 1-3.

Statistical analyses.—Statistical tests were performed in Statistica 7.0 (© StatSoft, Inc. 1984-2004 Tulsa). Animals found present at a spawning event, guarding offspring, resting at water, or resting on land during radio-tracking, were considered active by day. Nocturnal activity was deduced from overnight change in an animal's radio-tracked location, or overnight displacement of the stone placed on an animal's burrow. Mann-Whitney *U* tests

were performed to assess whether the sexes differed in the mean number of dates during summer that individuals were buried or active by day, and/or buried or active at night. Behavioural data were used from six males and seven females that were successfully radio-tracked in the day through Summers 2 and/or 3; and three males and seven females for which data on their nocturnal activity were obtained through Summer 3. Data presented are mean $\pm$ standard deviation.

We used generalized linear models (GLZs) to investigate animal activity, and population spawning in relation to meteorological variables. Diurnal or nocturnal animal activity was measured as the daily diurnal or nocturnal “active” (arc sine transformed) proportion of animals that were successfully radio-tracked on 129 days in Summers 1-3, or 31 days in Summer 3, respectively. Population spawning was measured as a binomial variable on 743 days in Summers 1-5. Diurnal animal activity and population spawning were examined in relation to study year, the amount of previous day’s rainfall (PDR), MaxT, MinT, mean day time RH, WS, and P (measured at 08h00 and 14h00), and DS. Nocturnal animal activity was examined in relation to the amount of daily rainfall (DR), MaxT, MinT, night time RH, WS, and P (measured at 20h00), DS, and ML.

We tested the predictor variables for multicollinearity using multiple regressions, and excluded predictors with a Variance Inflation Factor (VIF) > 2 , and/or a Tolerance (T) value < 0.5 . We therefore excluded RH (VIF = 3.1; $T = 0.3$) from the models of diurnal animal activity, DS (VIF = 2.4; $T = 0.4$), and MaxT (VIF = 2.3; $T = 0.4$) from the models of nocturnal animal activity; and RH (VIF = 3.6; $T = 0.3$) from the models of population

spawning. VIF and T values for the remaining predictor variables in the models are shown in Table 2. We assessed the utility of models of animal activity or population spawning based on a normal or binomial distribution, and an identity or logit link function, respectively. We corrected values of Akaike's Information Criterion (AIC) that were generated in Statistica, for over-dispersion of the data in all models (QAIC), and for small sample size in the models of diurnal or nocturnal animal activity (QAIC_c; Symonds & Moussalli 2010). For model averaging we used models with a corrected AIC value that differed by < 2 from that of the "best" model of population spawning, or diurnal or nocturnal animal activity (Symonds & Moussalli 2010). The importance of a specific predictor was estimated as the sum of the Akaike weights ($\sum w_i$) of all models of diurnal or nocturnal animal activity, or population spawning, which included that specific predictor (Symonds & Moussalli 2010).

We used a logistic regression with binomial errors to more accurately describe the relationship between PDR and population spawning, measured on 743 days in Summers 1-5. The duration of spawning events, or numbers of spawning males could not be examined in relation to meteorological variables using GLZs or step-wise multiple regressions, due to a small sample size ($n = 10$ observed spawning events), multicollinearity, and skewed variable distributions. Therefore, Spearman Rank correlations were used instead. Due to a small sample size ($n = 4$ summers), a Kendall Tau correlation was performed to determine whether annual numbers of spawning events were related to total summer rainfall through Summers 2-5. This method is more sensitive to some types of dependence, and deals better with ties between variables than the Spearman method (Kendall 1938).

RESULTS

Tracking.—Of the 30 transmitter-implanted animals, 10 males and 12 females were tracked post-release. Eight females were lost from the start, and one female died and another was lost for uncertain reasons shortly after release. The 10 males and 10 remaining females were each tracked to at least one burrow. Of these 20 animals, three females and two males were tracked for several days or weeks. Seven females and four males were tracked for a complete summer, and four males were tracked during two or three successive summers. We removed transmitters from five animals, but most animals were lost when their transmitters expired.

Male versus female activity.—Radio-tracked males were found on significantly more days in summer at water (10 ± 7 days, range: 4-18 days, $n = 6$ males tracked through Summer 2 and/or 3), than females (3 ± 2 days, range: 2-6 days, $n = 7$ females tracked through Summer 3, Mann-Whitney $U = 5.0$, $P = 0.02$). More specifically, males were found in summer on 4 ± 2 days at a spawning event (range: 3-6 days, $n = 6$ males), 1 ± 1 days guarding offspring (range: 0-2 days, $n = 4$ males), and 5 ± 5 days resting at water (range: 0-11 days, $n = 5$ males). Females were found in summer on significantly fewer days at a spawning event (2 ± 1 days, range: 2-3 days, $n = 7$ females, $U = 6.0$, $P = 0.03$), and on 1 ± 1 days resting at water (range: 0-3 days, $n = 2$ females, $U = 8.5$, $P = 0.07$). Only males were found in summer, on 2 ± 1 days resting on land (range: 0-4 days, $n = 5$). In Summer 3, males were

active on more nights (22 ± 5 nights, range: 16-26 nights, $n = 3$ males) than females (13 ± 5 nights, range: 9-20 nights, $n = 7$ females, $U = 2.0$, $P = 0.05$). Overall, males were active in the day and/or night on a greater cumulative number of dates in Summer 3 (27 ± 10 dates, range: 17-37 dates, $n = 3$ males), than females (14 ± 5 dates, range: 9-23 dates, $n = 7$ females, $U = 1.5$, $P = 0.04$).

Activity in relation to meteorological variables.—The relative importance (Σw_i) of each predictor of diurnal or nocturnal animal activity is shown in Table 3. Model averaging involved 62 models of diurnal animal activity, and 14 models of nocturnal animal activity. The coefficient estimate (β) and standard error (SE) obtained from model averaging, for the relationship between each predictor variable, and daily diurnal or nocturnal “active” proportions of successfully radio-tracked animals, is shown in Table 3.

PDR and DS were the first and second most important predictors in all 127 models of diurnal animal activity, and were included in 100 % and 52 % of the 62 “best candidate” models used for model averaging (Table 3). The coefficient estimates (Table 3) indicate that more animals were diurnally active (at water or on land) earlier in summer, and following heavier rainfall. MinT, night time WS, DR, and ML were, in descending order, the most important predictors in all 31 models of nocturnal animal activity, and were, respectively, included in 100 %, 86 %, 71 %, and 57 % of the 14 “best candidate” models used for model averaging (Table 3). The coefficient estimates (Table 3) indicate that more animals were nocturnally active (i.e., moved overland, and/or foraged) around full moon, after heavy rainfall, when cooler, and less windy conditions prevailed.

Spawning in relation to meteorological variables.—Daily rainfall in Diepsloot, and *P. adspersus* spawning at the study site in Summers 1-5, are depicted in Figures 1a-e, respectively. The logistic regression between PDR and population spawning, measured on 743 days in Summers 1-5, is shown in Fig. 1f. The coefficient estimate, SE, and *P* value was, respectively, 0.121, 0.014, and < 0.001 for PDR, and -4.254, 0.301, and < 0.001 for the regression intercept. The mean amount of rain recorded 24, 48 or 72 h prior to 25 spawning events that were observed, or revealed by offspring, was 40 ± 16 mm rain (min. = 20 mm), 45 ± 20 mm (min. = 22 mm), and 51 ± 25 mm (min. = 22 mm), respectively. Spawning occurred most frequently, in decreasing order, during December, January, and November (Fig. 2). The mean maximum count of males at 10 observed spawning events was 162 ± 148 males (range: 30-500 males), and the mean duration of 15 spawning events, of which 12 were observed and three were revealed by observation of one- or two-day old eggs, was 2 ± 1 days (range: 1-4 days).

The relative importance (Σw_i), coefficient estimate (β), and standard error (SE) of each predictor of population spawning, is shown in Table 3. Model averaging was based on 64 models. PDR and DS were, respectively, the first and second most important predictors in all 743 models of population spawning, and were included in 100 % and 50 % of the 64 “best candidate” models used for model averaging (Table 3). The coefficient estimates (Table 3) indicate that population spawning was more likely earlier in summer, and following heavier rain.

The Spearman Rank correlation between the duration of spawning events, or numbers of spawning males, and each of 11 different predictor variables, is shown in Table 4. The duration of spawning events was positively correlated with ML ($P = 0.05$), and the number of males at spawning events was positively correlated with the amount of previous day's rainfall ($P = 0.04$). Annually, there were 6 ± 1 spawning events (range: 4-7 spawning events, $n = 4$ summers), and estimated annual numbers of spawning events during Summers 2-5 were positively correlated with total summer rainfall (Kendall $T = 1.0$, $P = 0.04$, Fig. 3).

DISCUSSION

Male versus female activity.—*Pyxicephalus adspersus* is a difficult species to observe. Study animals cumulatively spent a month or less active during the six-month summer period. Other burrowing anurans inhabiting mesic or arid habitats exhibit similarly brief activity. For example, eastern spadefoots (*Scaphiopus holbrooki*) in Gainesville, Florida (Pearson 1955), and shoemaker frogs (*Neobatrachus sutor*) and orange-crowned toadlets (*Pseudophryne occidentalis*) in Western Australia (Thompson *et al.* 2003) spent, respectively, ca. 29 nights, and up to four days on 9 and 17 occasions, active in summer. During their brief activity, male *P. adspersus* that were radio-tracked for more than one season, evidently caught sufficient prey to sustain their large bulk through winter. This was possible, despite the degraded habitat, because *P. adspersus* preyed on termites, which were abundant on the site (CAY pers. obs.).

Certain activity differences between male and female *P. adspersus* might be due to their pronounced dimorphism in body size (Hayes & Licht 1992). Females avoid being terrestrially active during the day possibly because their greater surface-to-volume ratio increases their vulnerability to desiccation (Peters 1983). Males spend a greater number of nights active in summer because they visit breeding habitat more often, and because they probably need to spend more time foraging than females to satisfy their greater absolute metabolic requirements (Branch 1976; Paukstis & Reinbold 1984). Similarly, Ovaska (1992) claimed that females of the burrowing frog *Eleutherodactylus johnstonei*, moved longer distances than males overland, due to their larger size and greater energy requirements. In contrast, similar-sized (~ 50 g) male and female *Hoplobatrachus occipitalis* in a West African savanna, exhibited no difference in their timing of migrations to breeding sites, activity and home range areas, and growth during summer (Spieler & Linsenmair 1998).

Due to the more costly and limited production of ova relative to sperm (Van Beurden 1979; Reading 1988; Blem 1992), females of many anurans only spawn 1-3 times in a season, whereas males visit most spawning events to increase their reproductive success (e.g., Perrill & Daniel 1983; Lomen & Madsen 1986; Sinsch 1988; Barandun *et al.* 1997). *P. adspersus* appears to fit the same trend. If adult *P. adspersus* are present for only one day of a spawning event, females and males would, respectively, attend two (33%) and four (67%) of six mean spawning events per annum. Thus, assuming *P. adspersus* populations

have an adult sex ratio of 1:1, spawning events will usually appear male-biased in this species, as observed by Cook (1996).

Human disturbance at the Diepsloot dams accounts for why at least one radio-tracked male abandoned offspring (CAY pers. obs.), and likely explains why males spent one day on average in summer guarding offspring. In contrast, 109 males at Glen Austin Pan remained with their offspring for 21 ± 9.8 days (Cook *et al.* 2001).

Activity in relation to meteorological variables.—Radio-tracked males and females, respectively, spent 40 and 67% of their diurnal summer activity attending spawning events. Therefore, as with population spawning, a greater number of animals were active in the day after heavy rain, earlier in summer. Adult *P. adspersus* visit breeding sites after heavier rain because the risk of desiccation during overland migration between their burrows and breeding sites (Sjögren-Gulve 1998; Schwarzkopf & Alford 2002), and risk of evaporation and mortality of offspring at breeding sites (Kok *et al.* 1989; Cook *et al.* 2001) is probably reduced. Adults visit breeding sites, and spawn earlier in summer because adults may attend more spawning events per annum, adults and newly-metamorphosed froglets may accumulate larger energy reserves prior to winter, and/or the abundance of predators at breeding sites may increase over summer.

On nights with less wind, and/or increased moon light, greater proportions of animals were active because it was possibly easier to catch prey, and/or detect predators (Fitzgerald & Bider 1974; Penman *et al.* 2006). The influence of the moon or wind on anuran activity has

been considered in only a few studies. For example, Penman *et al.* (2006) showed that the activity of *Heleioporus australiacus* was related to rainfall, as well as temperature, humidity, and wind strength. A meta-analysis by Grant *et al.* (2009) revealed that various European amphibians exhibit mass migration and spawning around full moon. Some anurans are, however, terrestrially less active during full moon possibly because the risk of detection by predators is greater, and/or important prey is less active at this time (Church 1960; Ferguson 1960; Fitzgerald & Bider 1974; Goldberg & Schwalbe 2004).

Spawning in relation to meteorological variables.—*Pyxicephalus adspersus* often spawns after ca. 50 mm or more rainfall (Grobler 1972; Cook 1996; Du Preez & Cook 2004), but not always. At the Diepsloot site a minimum of 20 mm rain in 24 h could trigger spawning, which is equal to that reported for *P. adspersus* in the Free State Province (Kok *et al.* 1989), and for other explosive-breeding, burrowing anurans in mesic or arid habitats, e.g., *S. holbrooki* (Gosner & Black 1955). Although *P. adspersus* spawns readily in October, population spawning appears to be most common in November, December, and January (e.g., Balinsky 1969; Kok *et al.* 1989; Van Wyk *et al.* 1992; Cook 1996). This occurred at the study site because in October the artificial dams often remained dry, and by February and March, females, at least, had probably spent most or all of their ova.

Greater moon light prolonged spawning at the study site even though *P. adspersus* spawns predominantly during day light (Balinsky 1969; Channing *et al.* 1994). This was possibly because moon light facilitated foraging at night around the artificial dams, which enabled males to call, fight, and/or spawn over a longer period. We noticed that spawning was also

protracted when the weather was cool, windy, and/or overcast (CAY pers. obs.). However, due to a small sample size perhaps, no significant relationship emerged between spawning duration, and temperature, or WS. Greater numbers of males attended spawning events triggered by heavier rain because this possibly reduced the risk of desiccation during overland migration (Sjögren-Gulve 1998; Schwarzkopf & Alford 2002), and/or improved the probability of successful reproduction, particularly for young, weak, less experienced, and/or distantly buried males.

Cook *et al.* (2001) reported that *P. adspersus* breeds, on average, once in four years at a particular site. In contrast, *P. adspersus* at the Diepsloot study site spawned, on average, six times each summer. The reproductive success of *P. adspersus* may differ between large, natural pans, and small, artificial dams, but many spawning events probably go unnoticed due to the unpredictable, sporadic, and brief activity of this species. Therefore, *P. adspersus* could experience greater reproductive success than might be assumed. However, cohorts of larval *P. adspersus* often suffer high mortality caused by desiccation, and predation (Cook *et al.* 2001), and at high larval densities there is increased competition for food (Cook 1996), cannibalism (Grobler 1972), and possibly spread of disease.

Conservation implications.—Significant differences in the temporal and spatial use of aquatic and terrestrial habitat by adult male and female *P. adspersus* have important conservation implications for the species. Males spend significantly more time at breeding sites than females, and consequently, are more vulnerable to harvesting for human consumption, which is common in Limpopo Province (Du Preez & Cook 2004). Increased

mortality of males at breeding sites could adversely affect tadpole survival and juvenile recruitment due to paternal care of offspring in *P. adspersus* (Cook *et al.* 2001). In contrast, females spend virtually their entire lives in burrows situated significantly farther from breeding sites than those of males (Yetman & Ferguson in press). Females are consequently more vulnerable to encroaching land transformation (Du Preez & Cook 2004).

Pyxicephalus adspersus therefore requires effective protection of both aquatic and terrestrial habitat.

For estimation of *P. adspersus* population sizes, single counts of spawning adults are grossly inadequate because they will frequently result in severe underestimates. This was evident at our study site, where only a small proportion (10-50%) of the highest male count (i.e., 500 males in January of Summer 3) was present at most spawning events. Adult male *P. adspersus* remain relatively close to their breeding site within and among years (Yetman & Ferguson in press); therefore, frog movements are unlikely to account for the observed variation in numbers of males at spawning events. The great variation in numbers of spawning males at the Diepsloot dams obscured any trend in population size. Long term adult counts (exceeding 10 or 20 years; see Meyer *et al.* 2010) will, therefore, be necessary to detect real trends in the size of *P. adspersus* breeding populations. However, due to the unpredictable and brief activity of adults, it may be more practical to monitor, within certain areas, the number of aquatic sites where *P. adspersus* breeding occurs (Joseph *et al.* 2006). In this way large-scale trends can be detected for improved conservation assessment and management of *P. adspersus* at regional, provincial and national scales (Lindenmayer & Likens 2010).

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Table 1. Assignment of days since full moon (DFM) to one of eight lunar phases, ranked from 0-4 depending on the amount of moon light (adapted from Grant *et al.* 2009).

	Full moon	Waning gibbous	3 rd quarter	Waning crescent	New moon	Waxing crescent	1st quarter	Waxing gibbous
Lunar phase	1	2	3	4	5	6	7	8

DFM	28, 29, 0, 1	2, 3, 4, 5	6, 7, 8	9, 10, 11, 12	13, 14, 15, 16	17, 18, 19, 20	21, 22, 23	24, 25, 26, 27
Rank	4	3	2	1	0	1	2	3

Table 2. Variance Inflation Factor (VIF) and Tolerance (T) values for predictor variables used in generalized linear models of the diurnal, nocturnal, or spawning activity of *Pyxicephalus adspersus* at the Diepsloot study site. The values were obtained from multiple regressions performed after certain variables with a $VIF > 2$ and/or $T < 0.5$, were excluded (excl.) to reduce multicollinearity.

	Diurnal activity		Nocturnal activity		Spawning	
	VIF	T	VIF	T	VIF	T
Study year	1.2	0.8	-	-	1.0	1.0
Day of season	1.4	0.7	excl.	excl.	1.2	0.8
Previous day's rain	1.3	0.8	-	-	1.2	0.8
Daily rain	-	-	1.2	0.8	-	-
Max. temperature	1.3	0.8	excl.	excl.	1.5	0.7
Min. temperature	1.3	0.8	1.7	0.6	1.4	0.7
Wind speed	1.1	0.9	1.1	0.9	1.2	0.9
Relative humidity	excl.	excl.	1.4	0.7	excl.	excl.
Atmospheric pressure	1.1	0.9	1.5	0.7	1.2	0.8
Moon light	-	-	1.2	0.8	1.0	1.0

Table 3. Percent (%) inclusion, relative importance (Σw_i), coefficient estimate (β), and standard error (SE) of predictor variables used in generalized linear models of the diurnal, nocturnal, or spawning activity of *Pyxicephalus adspersus* at the Diepsloot study site. Percent inclusion pertains to the 62, 14, or 64 “best” models of diurnal, nocturnal, or spawning activity used for model averaging. Σw_i represents the sum of the Akaike weights of every diurnal, nocturnal, or spawning activity model (max. $n = 127, 31$, or 743 models, respectively) that involved a specific predictor.

	Diurnal animal activity				Nocturnal animal activity				Population spawning			
	%	Σw_i	β	SE	%	Σw_i	β	SE	%	Σw_i	β	SE
Intercept	-	-	0.672	0.003	-	-	3.687	0.078	-	-	54.446	0.122
Study year	48	0.46	-0.012	4×10^{-5}	-	-	-	-	50	0.49	-0.056	2×10^{-4}
Day of season	52	0.58	-0.001	2×10^{-6}	-	-	-	-	50	0.60	-0.012	2×10^{-5}
Previous day's rain	100	0.87	0.009	5×10^{-6}	-	-	-	-	100	0.93	0.122	6×10^{-5}
Daily rain	-	-	-	-	71	0.63	0.006	9×10^{-5}	-	-	-	-
Max. temperature	50	0.51	-0.005	1×10^{-5}	-	-	-	-	50	0.50	-0.007	7×10^{-5}
Min. temperature	50	0.54	-0.016	3×10^{-5}	100	0.76	-0.061	5×10^{-4}	50	0.50	0.054	2×10^{-4}
Wind speed	48	0.45	3×10^{-5}	7×10^{-6}	86	0.63	-0.061	7×10^{-4}	50	0.49	-0.014	9×10^{-5}
Relative humidity	-	-	-	-	43	0.47	7×10^{-4}	2×10^{-5}	-	-	-	-
Atmospheric pressure	48	0.45	-4×10^{-5}	3×10^{-6}	36	0.47	-0.003	9×10^{-5}	50	0.51	-0.068	1×10^{-4}
Moon light	-	-	-	-	57	0.54	0.043	9×10^{-4}	-	-	-	-

Table 4. Spearman Rank correlation between the duration of, or maximum number of males counted at, 10 observed *Pyxicephalus adspersus* spawning events, and various meteorological variables in Diepsloot. Not significant = n.s., * $P < 0.05$.

	Spawning duration Max. no. of males			
	r_s	P	r_s	P
Study year	-0.52	n.s.	0.12	n.s.
Day of season ¹	-0.10	n.s.	0.19	n.s.
Previous day's rain ¹	0.03	n.s.	0.65	*
Total rain ²	0.43	n.s.	-0.11	n.s.
Max. temperature ³	0.02	n.s.	-0.18	n.s.
Min. temperature ³	0.04	n.s.	0.04	n.s.
Wind speed ³	0.28	n.s.	0.21	n.s.
Relative humidity ³	-0.27	n.s.	-0.02	n.s.
Atmospheric pressure ³	-0.08	n.s.	0.41	n.s.
Moon light ¹	0.64	*	0.06	n.s.
Max. no. of males ²	0.34	n.s.	-	-
Duration of spawning	-	-	0.34	n.s.

¹ For the first day of spawning.

² Over the full duration of spawning.

³ Mean of daily values during spawning.

A Summer 1 (2003/2004)

D Summer 4 (2006/2007)

B Summer 2 (2004/2005)

E Summer 5 (2007/2008)

C Summer 3 (2005/2006)

F Logistic regression

Figure 1. Daily rainfall in Diepsloot, Gauteng Province, South Africa, and known *Pyxicephalus adspersus* spawning activity at the study site during Summers 1-5, shown in A-E, respectively. Spawning events represented by vertical bars were observed. Those indicated by ✓ were revealed by the presence of eggs, tadpoles, and/or froglets. ✕ indicates when spawning did not occur after heavy rain. (F) Logistic regression between the amount of previous day's rainfall, and *P. adspersus* spawning at the site, measured on 743 days in Summers 1-5.

Figure 2. The number of recorded *Pyxicephalus adspersus* spawning events at the Diepsloot study site in different months during Summers 1-5. Thirteen spawning events were observed, and 12 were revealed by the presence of eggs, tadpoles and/or froglets.

Figure 3. Kendall Tau correlation between total summer rainfall, and annual estimated numbers of *Pyxicephalus adspersus* spawning events during four summers at the Diepsloot study site.